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(54) Title: **HYPERSENSITIVE RESPONSE ELICITOR FRAGMENTS WHICH ARE ACTIVE BUT DO NOT ELICIT A HYPERSENSITIVE RESPONSE**

(57) Abstract

The present invention is directed to isolated active fragments of a hypersensitive response elicitor protein or polypeptide which fragment does not elicit a hypersensitive response in plants. Also disclosed are isolated DNA molecules which encode such fragments. Isolated fragments of hypersensitive response elicitor proteins or polypeptides in accordance with the present invention and the isolated DNA molecules that encode them have the following activities: imparting disease resistance to plants, enhancing plant growth, and/or controlling insects on plants. This can be achieved by applying the fragments of a hypersensitive response elicitor in a non-infectious form to plants or plant seeds under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds. Alternatively, transgenic plants or plant seeds transformed with a DNA molecule encoding the fragment can be provided and the transgenic plants or plants resulting from the transgenic plant seeds are grown under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

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HYPERSENSITIVE RESPONSE ELICITOR FRAGMENTS WHICH ARE ACTIVE BUT DO NOT ELICIT A HYPERSENSITIVE RESPONSE

This application claims benefit of U.S. Provisional Patent Application
5 Serial No. 60/103,050, filed October 5, 1998.

FIELD OF THE INVENTION

The present invention relates to active fragments of a hypersensitive
10 response elicitor which fragments do not elicit a hypersensitive response.

BACKGROUND OF THE INVENTION

Interactions between bacterial pathogens and their plant hosts generally
15 fall into two categories: (1) compatible (pathogen-host), leading to intercellular bacterial growth, symptom development, and disease development in the host plant; and (2) incompatible (pathogen-nonhost), resulting in the hypersensitive response, a particular type of incompatible interaction occurring, without progressive disease symptoms. During compatible interactions on host plants, bacterial populations
20 increase dramatically and progressive symptoms occur. During incompatible interactions, bacterial populations do not increase, and progressive symptoms do not occur.

The hypersensitive response is a rapid, localized necrosis that is associated with the active defense of plants against many pathogens (Kiraly, Z.,
25 "Defenses Triggered by the Invader: Hypersensitivity," pages 201-224 in: Plant Disease: An Advanced Treatise, Vol. 5, J.G. Horsfall and E.B. Cowling, ed. Academic Press New York (1980); Klement, Z., "Hypersensitivity," pages 149-177 in: Phytopathogenic Prokaryotes, Vol. 2, M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The hypersensitive response elicited by bacteria is readily observed as a tissue collapse if high concentrations ($\geq 10^7$ cells/ml) of a limited host-range pathogen like *Pseudomonas syringae* or *Erwinia amylovora* are infiltrated into the leaves of nonhost plants (necrosis occurs only in isolated plant cells at lower levels of inoculum) (Klement, Z., "Rapid Detection of Pathogenicity of Phytopathogenic Pseudomonads," Nature 199:299-300; Klement, et al.,

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“Hypersensitive Reaction Induced by Phytopathogenic Bacteria in the Tobacco Leaf,” Phytopathology 54:474-477 (1963); Turner, et al., “The Quantitative Relation Between Plant and Bacterial Cells Involved in the Hypersensitive Reaction,” Phytopathology 64:885-890 (1974); Klement, Z., “Hypersensitivity,” pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The capacities to elicit the hypersensitive response in a nonhost and be pathogenic in a host appear linked. As noted by Klement, Z., “Hypersensitivity,” pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York, these pathogens also cause 10 physiologically similar, albeit delayed, necroses in their interactions with compatible hosts. Furthermore, the ability to produce the hypersensitive response or pathogenesis is dependent on a common set of genes, denoted *hrp* (Lindgren, P.B., et al., “Gene Cluster of *Pseudomonas syringae* pv. ‘phaseolicola’ Controls Pathogenicity of Bean Plants and Hypersensitivity on Nonhost Plants,” J. Bacteriol. 168:512-22 (1986); 15 Willis, D.K., et al., “*hrp* Genes of Phytopathogenic Bacteria,” Mol. Plant-Microbe Interact. 4:132-138 (1991)). Consequently, the hypersensitive response may hold clues to both the nature of plant defense and the basis for bacterial pathogenicity.

The *hrp* genes are widespread in Gram-negative plant pathogens, where they are clustered, conserved, and in some cases interchangeable (Willis, D.K., et al., “*hrp* Genes of Phytopathogenic Bacteria,” Mol. Plant-Microbe Interact. 4:132-20 138 (1991); Bonas, U., “*hrp* Genes of Phytopathogenic Bacteria,” pages 79-98 in: Current Topics in Microbiology and Immunology: Bacterial Pathogenesis of Plants and Animals - Molecular and Cellular Mechanisms, J.L. Dangl, ed. Springer-Verlag, Berlin (1994)). Several *hrp* genes encode components of a protein secretion pathway 25 similar to one used by *Yersinia*, *Shigella*, and *Salmonella* spp. to secrete proteins essential in animal diseases (Van Gijsegem, et al., “Evolutionary Conservation of Pathogenicity Determinants Among Plant and Animal Pathogenic Bacteria,” Trends Microbiol. 1:175-180 (1993)). In *E. amylovora*, *P. syringae*, and *P. solanacearum*, *hrp* genes have been shown to control the production and secretion of glycine-rich, 30 protein elicitors of the hypersensitive response (He, S.Y., et al. “*Pseudomonas Syringae* pv. *Syringae* HarpinPss: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants,” Cell 73:1255-1266 (1993), Wei, Z.-H.,

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et al., "HrpI of *Erwinia amylovora* Functions in Secretion of Harpin and is a Member of a New Protein Family," J. Bacteriol. 175:7958-7967 (1993); Arlat, M. et al.

"PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J.

5 J. 13:543-553 (1994)).

The first of these proteins was discovered in *E. amylovora* Ea321, a bacterium that causes fire blight of rosaceous plants, and was designated harpin (Wei, Z.-M., et al, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992)). Mutations in the encoding 10 *hrpN* gene revealed that harpin is required for *E. amylovora* to elicit a hypersensitive response in nonhost tobacco leaves and incite disease symptoms in highly susceptible pear fruit. The *P. solanacearum* GMI1000 PopA1 protein has similar physical properties and also elicits the hypersensitive response in leaves of tobacco, which is not a host of that strain (Arlat, et al. "PopA1, a Protein Which Induces a 15 Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-53 (1994)). However, *P. solanacearum* *popA* mutants still elicit the hypersensitive response in tobacco and incite disease in tomato. Thus, the role of these glycine-rich hypersensitive response elicitors can vary widely among Gram-negative plant pathogens.

20 Other plant pathogenic hypersensitive response elicitors have been isolated, cloned, and sequenced. These include: *Erwinia chrysanthemi* (Bauer, et. al., "Erwinia chrysanthemi Harpin_{Ech}: Soft-Rot Pathogenesis," MPMI 8(4): 484-91 (1995)); *Erwinia carotovora* (Cui, et. al., "The RsmA⁻ Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrpN*_{Ecc} and Elicit a Hypersensitive 25 Reaction-like Response in Tobacco Leaves," MPMI 9(7): 565-73 (1996)); *Erwinia stewartii* (Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microb. Inter. July 14-19, 1996 and Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc. July 27-31, 1996); and *Pseudomonas 30 syringae* pv. *syringae* (WO 94/26782 to Cornell Research Foundation, Inc.).

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The present invention seeks to identify fragments of hypersensitive response elicitor proteins or polypeptides, which fragments do not elicit a hypersensitive response but are active when utilized in conjunction with plants.

5

SUMMARY OF THE INVENTION

The present invention is directed to isolated fragments of an *Erwinia* hypersensitive response elicitor protein or polypeptide which fragments do not elicit a hypersensitive response in plants but are otherwise active when utilized in 10 conjunction with plants. Also disclosed are isolated DNA molecules which encode such fragments.

The fragments of hypersensitive response elicitors according to the present invention have the following activity when utilized in conjunction with plants: imparting disease resistance to plants, enhancing plant growth and/or controlling 15 insects. This involves applying the fragments in a non-infectious form to plants or plant seeds under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

As an alternative to applying the fragments to plants or plant seeds in order to impart disease resistance, to enhance plant growth, and/or to control insects 20 on plants, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a fragment of a hypersensitive response elicitor protein or polypeptide in accordance with the present invention and growing the plant under conditions effective to impart disease resistance, to enhance plant growth, and/or to control 25 insects in the plants or plants grown from the plant seeds. Alternatively, a transgenic plant seed transformed with the DNA molecule encoding such a fragment can be provided and planted in soil. A plant is then propagated under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

30

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows truncated proteins of the hypersensitive response elicitor protein or polypeptide.

5 Figure 2 shows a list of synthesized oligonucleotide primers for construction of truncated harpin proteins. N represents the N-terminus (5' region), and C represents the C-terminus (3' region). The primers correspond to the indicated sequence identification numbers for the present application: N1 (SEQ. ID. No. 1), N76 (SEQ. ID. No. 2), N99 (SEQ. ID. No. 3), N105 (SEQ. ID. No. 4), N110 (SEQ. 10 ID. No. 5), N137 (SEQ. ID. No. 6), N150 (SEQ. ID. No. 7), N169 (SEQ. ID. No. 8), N210 (SEQ. ID. No. 9), N267 (SEQ. ID. No. 10), N343 (SEQ. ID. No. 11), C75 (SEQ. ID. No. 12), C104 (SEQ. ID. No. 13), C168 (SEQ. ID. No. 14), C180 (SEQ. ID. No. 15), C204 (SEQ. ID. No. 16), C209 (SEQ. ID. No. 17), C266 (SEQ. ID. No. 18), C342 (SEQ. ID. No. 19), and C403 (SEQ. ID. No. 20).

15

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to isolated fragments of a hypersensitive response elicitor protein or polypeptide where the fragments do not 20 elicit a hypersensitive response but have other activity in plants. Also disclosed are DNA molecules encoding such fragments as well as expression systems, host cells, and plants containing such molecules. Uses of the fragments themselves and the DNA molecules encoding them are disclosed.

The fragments of hypersensitive response elicitor polypeptides or 25 proteins according to the present invention are derived from hypersensitive response elicitor polypeptides or proteins of a wide variety of fungal and bacterial pathogens. Such polypeptides or proteins are able to elicit local necrosis in plant tissue contacted by the elicitor. Examples of suitable bacterial sources of polypeptide or protein elicitors include *Erwinia*, *Pseudomonas*, and *Xanthomonas* species (e.g., the 30 following bacteria: *Erwinia amylovora*, *Erwinia chrysanthemi*, *Erwinia stewartii*, *Erwinia carotovora*, *Pseudomonas syringae*, *Pseudomonas solancearum*, *Xanthomonas campestris*, and mixtures thereof).

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An example of a fungal source of a hypersensitive response elicitor protein or polypeptide is *Phytophthora*. Suitable species of *Phytophthora* include *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora*.

5 The hypersensitive response elicitor polypeptide or protein from *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 21 as follows:

10	Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser	1	5	10	15
	Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser	20	25	30	
	Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr	35	40	45	
15	Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu	50	55	60	
	Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser	65	70	75	80
20	Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys	85	90	95	
	Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp	100	105	110	
	Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln	115	120	125	
25	Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met	130	135	140	
	Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly	145	150	155	160
30	Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly	165	170	175	
	Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu	180	185	190	
	Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala	195	200	205	
35	Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val	210	215	220	
	Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp	225	230	235	240

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15 This hypersensitive response elicitor polypeptide or protein has a molecular weight of
34 kDa, is heat stable, has a glycine content of greater than 16%, and contains
substantially no cysteine. The *Erwinia chrysanthemi* hypersensitive response elicitor
polypeptide or protein is encoded by a DNA molecule having a nucleotide sequence
20 corresponding to SEQ. ID. No. 22 as follows:

CGATTTTACC	CGGGTGAACG	TGCTATGACC	GACAGCATCA	CGGTATTGCA	CACCGTTACG	60	
GCGTTTATGG	CCGCGATGAA	CCGGCATCAG	GCGGCGCGCT	GGTCGCCGCA	ATCCGGCGTC	120	
GATCTGGTAT	TTCAGTTGG	GGACACCGGG	CGTGAACCTCA	TGATGCAGAT	TCAGCCGGGG	180	
25	CAGCAATATC	CCGGCATGTT	GCGCACGCTG	CTCGCTCGTC	GTTATCAGCA	GGCGGCAGAG	240
	TGCGATGGCT	GCCATCTGTG	CCTGAACGGC	AGCGATGTAT	TGATCCTCTG	GTGGCCGCTG	300
	CCGTCGGATC	CCGGCAGTTA	TCCGCAGGTG	ATCGAACGTT	TGTTTGAACT	GGCGGGAAATG	360
	ACGTTGCCGT	CGCTATCCAT	AGCACCGACG	GCGCGTCCGC	AGACAGGGAA	CGGACGCGCC	420
	CGATCATTAA	GATAAAGGCG	GCTTTTTTA	TTGCAAAACG	GTAACGGTGA	GGAACCGTTT	480
30	CACCGTCGGC	GTCACTCAGT	AACAAGTATC	CATCATGATG	CCTACATCGG	GATCGGCGTG	540
	GGCATCCGTT	GCAGATACTT	TTGCGAACAC	CTGACATGAA	TGAGGAAACG	AAATTATGCA	600
	AATTACGATC	AAAGCGCACA	TCGGCGGTGA	TTTGGGCGTC	TCCGGTCTGG	GGCTGGGTGC	660
	TCAGGGACTG	AAAGGACTGA	ATTCCGCGGC	TTCATCGCTG	GGTCCAGCG	TGGATAAACT	720
	GAGCAGCACC	ATCGATAAGT	TGACCTCCGC	GCTGACTTCG	ATGATGTTG	GCGGCGCGCT	780
35	GGCGCAGGGG	CTGGGCGCCA	GCTCGAAGGG	GCTGGGGATG	AGCAATCAAC	TGGGCCAGTC	840

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	TTTCGGCAAT GGCGCGCAGG GTGCGAGCAA CCTGCTATCC GTACCGAAAT CCGGCGGCGA	900
	TGCGTTGTCA AAAATGTTTG ATAAAGCGCT GGACGATCTG CTGGGTCATG ACACCGTGAC	960
	CAAGCTGACT AACCAAGAGCA ACCAACTGGC TAATTCAATG CTGAACGCCA GCCAGATGAC	1020
	CCAGGGTAAT ATGAATGCGT TCGGCAGCGG TGTGAACAAAC GCACGTGCGT CCATTCTCGG	1080
5	CAACGGTCTC GGCCAGTCGA TGAGTGGCTT CTCTCAGCCT TCTCTGGGG CAGGCGGCTT	1140
	GCAGGGCCTG AGCGGCGCGG GTGCATTCAA CCAGTTGGGT AATGCCATCG GCATGGGCGT	1200
	GGGGCAGAAT GCTGCGCTGA GTGCGTTGAG TAACGTCAGC ACCCACGTAG ACGGTAACAA	1260
	CCGCCACTTT GTAGATAAAG AAGATCGCGG CATGGCGAAA GAGATCGGCC AGTTTATGGA	1320
	TCAGTATCCG GAAATATTG GAAACCGGA ATACCAGAAA GATGGCTGGA GTTCGCCGAA	1380
10	GACGGACGAC AAATCCTGGG CTAAGCGCT GAGTAAACCG GATGATGACG GTATGACCGG	1440
	CGCCAGCATG GACAAATTCC GTCAGGCGAT GGGTATGATC AAAAGCGCGG TGGCGGGTGA	1500
	TACCGGCAAT ACCAACCTGA ACCTGCGTGG CGCGGGCGGT GCATCGCTGG GTATCGATGC	1560
	GGCTGTCGTC GGCGATAAAA TAGCCAACAT GTCGCTGGGT AAGCTGGCCA ACGCCTGATA	1620
	ATCTGTGCTG GCCTGATAAA CGGGAAACGA AAAAGAGAC GGGGAAGCCT GTCTTTTC	1680
15	TTATTATGCG GTTTATGCGG TTACCTGGAC CGGTTAATCA TCGTCATCGA TCTGGTACAA	1740
	ACGCACATTT TCCCGTTCAT TCGCGTCGTT ACGCGCCACA ATCGCGATGG CATCTCCTC	1800
	GTGCGTCAGA TTGCGCGGCT GATGGGAAC GCCGGGTGGA ATATAGAGAA ACTCGCCGGC	1860
	CAGATGGAGA CACGCTGCG ATAAATCTGT GCCGTAACGT GTTTCTATCC GCCCCTTTAG	1920
	CAGATAGATT GCGGTTCGT AATCAACATG GTAATGCGGT TCCGCCTGTG CGCCGGCGG	1980
20	GATCACCACA ATATTCAAG AAAGCTGTCT TGCACCTACC GTATCGCGGG AGATACCGAC	2040
	AAAATAGGGC AGTTTTGCG TGGTATCCGT GGGGTGTTCC GGCCTGACAA TCTTGAGTTG	2100
	GTTCGTCATC ATCTTCTCC ATCTGGCGA CCTGATCGGT T	2141

25 The hypersensitive response elicitor polypeptide or protein derived
from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID.
No. 23 as follows:

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	Asn	Ala	Gly	Leu	Gly	Gly	Asn	Ser	Ala	Leu	Gly	Leu	Gly	Gly	Asn	
			35				40			45						
	Gln	Asn	Asp	Thr	Val	Asn	Gln	Leu	Ala	Gly	Leu	Leu	Thr	Gly	Met	Met
			50				55			60						
5	Met	Met	Met	Ser	Met	Met	Gly	Gly	Gly	Gly	Leu	Met	Gly	Gly	Leu	
	65					70			75		80					
	Gly	Gly	Gly	Leu	Gly	Asn	Gly	Leu	Gly	Gly	Ser	Gly	Gly	Leu	Gly	Glu
				85				90			95					
10	Gly	Leu	Ser	Asn	Ala	Leu	Asn	Asp	Met	Leu	Gly	Gly	Ser	Leu	Asn	Thr
				100				105			110					
	Leu	Gly	Ser	Lys	Gly	Gly	Asn	Asn	Thr	Thr	Ser	Thr	Thr	Asn	Ser	Pro
				115				120			125					
	Leu	Asp	Gln	Ala	Leu	Gly	Ile	Asn	Ser	Thr	Ser	Gln	Asn	Asp	Asp	Ser
				130			135			140						
15	Thr	Ser	Gly	Thr	Asp	Ser	Thr	Ser	Asp	Ser	Ser	Asp	Pro	Met	Gln	Gln
				145			150			155			160			
	Leu	Leu	Lys	Met	Phe	Ser	Glu	Ile	Met	Gln	Ser	Leu	Phe	Gly	Asp	Gly
				165			170			175						
20	Gln	Asp	Gly	Thr	Gln	Gly	Ser	Ser	Ser	Gly	Gly	Lys	Gln	Pro	Thr	Glu
				180			185			190						
	Gly	Glu	Gln	Asn	Ala	Tyr	Lys	Lys	Gly	Val	Thr	Asp	Ala	Leu	Ser	Gly
				195			200			205						
	Leu	Met	Gly	Asn	Gly	Leu	Ser	Gln	Leu	Leu	Gly	Asn	Gly	Leu	Gly	
				210			215			220						
25	Gly	Gly	Gln	Gly	Gly	Asn	Ala	Gly	Thr	Gly	Leu	Asp	Gly	Ser	Ser	Leu
				225			230			235			240			
	Gly	Gly	Lys	Gly	Leu	Gln	Asn	Leu	Ser	Gly	Pro	Val	Asp	Tyr	Gln	Gln
				245			250			255						
30	Leu	Gly	Asn	Ala	Val	Gly	Thr	Gly	Ile	Gly	Met	Lys	Ala	Gly	Ile	Gln
				260			265			270						
	Ala	Leu	Asn	Asp	Ile	Gly	Thr	His	Arg	His	Ser	Ser	Thr	Arg	Ser	Phe
				275			280			285						
	Val	Asn	Lys	Gly	Asp	Arg	Ala	Met	Ala	Lys	Glu	Ile	Gly	Gln	Phe	Met
				290			295			300						
35	Asp	Gln	Tyr	Pro	Glu	Val	Phe	Gly	Lys	Pro	Gln	Tyr	Gln	Lys	Gly	Pro
				305			310			315			320			
	Gly	Gln	Glu	Val	Lys	Thr	Asp	Asp	Lys	Ser	Trp	Ala	Lys	Ala	Leu	Ser
				325			330			335						
	Lys	Pro	Asp	Asp	Asp	Gly	Met	Thr	Pro	Ala	Ser	Met	Glu	Gln	Phe	Asn

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340

345

350

Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn
 355 360 365

5 Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp
 370 375 380

Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu
 385 390 395 400

Gly Ala Ala

This hypersensitive response elicitor polypeptide or protein has a molecular weight of
 10 about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10
 minutes. This hypersensitive response elicitor polypeptide or protein has substantially
 no cysteine. The hypersensitive response elicitor polypeptide or protein derived from
Erwinia amylovora is more fully described in Wei, Z.-M., R. J. Laby, C. H. Zumoff,
 D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, "Harpin, Elicitor of the
 15 Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*,"
Science 257:85-88 (1992), which is hereby incorporated by reference. The DNA
 molecule encoding this polypeptide or protein has a nucleotide sequence
 corresponding to SEQ. ID. No. 24 as follows:

20	AAGCTTCGGC ATGGCACGTT TGACCGTTGG GTCGGCAGGG TACGTTTGAA TTATTCATAA	60
	GAGGAATACG TTATGAGTCT GAATACAAGT GGGCTGGGAG CGTCAACGAT GCAAATTTCT	120
	ATCGGCGGTG CGGGCGGAAA TAACGGGTTG CTGGGTACCA GTCGCCAGAA TGCTGGGTTG	180
	GGTGGCAATT CTGCACTGGG GCTGGGCGGC GGTAAATCAAA ATGATAACCGT CAATCAGCTG	240
	GCTGGCTTAC TCACCGGCAT GATGATGATG ATGAGCATGA TGGGCGGTGG TGGGCTGATG	300
25	GGCGGTGGCT TAGGCGGTGG CTTAGGTAAAT GGCTTGGGTG GCTCAGGTGG CCTGGGCGAA	360
	GGACTGTCGA ACGCGCTGAA CGATATGTTA GGCGGTTCGC TGAACACGCT GGGCTCGAAA	420
	GGCGGCAACA ATACCACTTC AACAAACAAAT TCCCCGCTGG ACCAGGCGCT GGGTATTAAC	480
	TCAACGTCCC AAAACGACGA TTCCACCTCC GGCACAGATT CCACCTCAGA CTCCAGCGAC	540
	CCGATGCAGC AGCTGCTGAA GATGTTCAAGC GAGATAATGC AAAGCCTGTT TGGTGATGGG	600
30	CAAGATGGCA CCCAGGGCAG TTCCTCTGGG GGCAAGCAGC CGACCGAAGG CGAGCAGAAC	660
	GCCTATAAAA AAGGAGTCAC TGATGCGCTG TCGGGCCTGA TGGGTAATGG TCTGAGCCAG	720
	CTCCTTGGCA ACGGGGGACT GGGAGGTGGT CAGGGCGGTA ATGCTGGCAC GGGTCTTGAC	780
	GGTTCGTCGC TGGGCGGCAA AGGGCTGCAA AACCTGAGCG GGCGCGTGGGA CTACCAGCAG	840

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TTAGGTAACG	CCGTGGGTAC	CGGTATCGGT	ATGAAAGCGG	GCATTCAAGGC	GCTGAATGAT	900	
ATCGGTACGC	ACAGGCACAG	TTCAACCCGT	TCTTTCGTCA	ATAAAGGCGA	TCGGGCGATG	960	
GCGAAGGAAA	TCGGTCAGTT	CATGGACCAG	TATCCTGAGG	TGTTTGGCAA	GCCGCAGTAC	1020	
CAGAAAGGCC	CGGGTCAGGA	GGTGAAAACC	GATGACAAAT	CATGGCAAA	AGCACTGAGC	1080	
5	AAGCCAGATG	ACGACGGAAT	GACACCAGCC	AGTATGGAGC	AGTTCAACAA	AGCCAAGGGC	1140
ATGATCAAAA	GGCCCATGGC	GGGTGATACC	GGCAACGGCA	ACCTGCAGGC	ACCGGGTGCC	1200	
GGTGGTTCTT	CGCTGGGTAT	TGATGCCATG	ATGGCCGGTG	ATGCCATTAA	CAATATGGCA	1260	
CTTGGCAAGC	TGGGCGCGGC	TTAAGCTT				1288	

10

Another potentially suitable hypersensitive response elicitor from *Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,927, which is hereby incorporated by reference. The protein is encoded by a DNA molecule having a nucleic acid sequence of SEQ. ID. No. 25 as follows:

15

ATGTCAATT	TTACGTTAA	CAACAATACC	TCGTCCTCGC	CGGGTCTGTT	CCAGTCCGGG	60
GGGGACAACG	GGCTTGGTGG	TCATAATGCA	AATTCTGCGT	TGGGGCAACA	ACCCATCGAT	120

20

CGGCAAACCA	TTGAGCAAAT	GGCTCAATT	TTGGCGGAAC	TGTTAAAGTC	ACTGCTATCG	180
CCACAATCAG	GTAATGCGGC	AACCGGAGCC	GGTGGCAATG	ACCAAGACTAC	AGGAGTTGGT	240

25

AACGCTGGCG	GCCTGAACGG	ACGAAAAGGC	ACAGCAGGAA	CCACTCCGCA	GTCTGACAGT	300
CAGAACATGC	TGAGTGAGAT	GGGCAACAAC	GGGCTGGATC	AGGCCATCAC	GCCCGATGGC	360

30

CAGGGCGGGCG	GGCAGATCGG	CGATAATCCT	TTACTGAAAG	CCATGCTGAA	GCTTATTGCA	420
CGCATGATGG	ACGGCCAAAG	CGATCAGTTT	GGCCAACCTG	GTACGGCAA	CAACAGTGCC	480

35

TCTTCCGGTA	CTTCTTCATC	TGGCGGTCC	CCTTTTAACG	ATCTATCAGG	GGGGAAAGGCC	540
CCTTCCGGCA	ACTCCCCCTTC	CGGCAACTAC	TCTCCCGTCA	GTACCTTCTC	ACCCCCATCC	600

ACGCCAACGT	CCCCTACCTC	ACCGCTTGAT	TTCCCTTCTT	CTCCCACCAA	AGCAGCCGGG	660
GGCAGCACGC	CGGTAACCGA	TCATCCTGAC	CCTGTTGGTA	GCGCGGGCAT	CGGGGCGCGGA	720

40

AATTCGGTGG	CCTTCACCAAG	CGCCGGCGCT	AATCAGACGG	TGCTGCATGA	CACCATTACC	780
GTGAAAGCGG	GTCAGGTGTT	TGATGGCAA	GGACAAACCT	TCACCGCCGG	TTCAGAATTA	840

45

GGCGATGGCG	GCCAGTCTGA	AAACCAGAAA	CCGCTGTTA	TACTGGAAGA	CGGTGCCAGC	900
CTGAAAAACG	TCACCATGGG	CGACGACGGG	GCGGATGGTA	TTCATTTA	CGGTGATGCC	960

AAAATAGACA	ATCTGCACGT	CACCAACGTG	GGTGAGGACG	CGATTACCGT	TAAGCCAAAC	1020	
50	AGCGCGGGCA	AAAAATCCCA	CGTTGAAATC	ACTAACAGTT	CCTTCGAGCA	CGCCTCTGAC	1080

AAGATCCTGC	AGCTGAATGC	CGATACTAAC	CTGAGCGTTG	ACAACGTGAA	GGCCAAAGAC	1140
------------	------------	------------	------------	------------	------------	------

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5	TTTGGTACTT TTGTACGCAC TAACGGCGGT CAACAGGGTA ACTGGGATCT GAATCTGAGC	1200
	CATATCAGCG CAGAAGACGG TAAGTTCTCG TTCGTTAAAAA GCGATAGCGA GGGGCTAAAC	1260
	GTCAATACCA GTGATATCTC ACTGGGTGAT GTTGAAAACC ACTACAAAGT GCCGATGTCC	1320
	GCCAACCTGA AGGTGGCTGA ATGA	1344

10

See GenBank Accession No. U94513. The isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 26 as follows:

15	Met Ser Ile Leu Thr Leu Asn Asn Asn Thr Ser Ser Ser Pro Gly Leu	
	1 5 10 15	
	Phe Gln Ser Gly Gly Asp Asn Gly Leu Gly Gly His Asn Ala Asn Ser	
	20 25 30	
20	Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala	
	35 40 45	
25	Gln Leu Leu Ala Glu Leu Leu Lys Ser Leu Leu Ser Pro Gln Ser Gly	
	50 55 60	
	Asn Ala Ala Thr Gly Ala Gly Gly Asn Asp Gln Thr Thr Gly Val Gly	
	65 70 75 80	
30	Asn Ala Gly Gly Leu Asn Gly Arg Lys Gly Thr Ala Gly Thr Thr Pro	
	85 90 95	
	Gln Ser Asp Ser Gln Asn Met Leu Ser Glu Met Gly Asn Asn Gly Leu	
	100 105 110	
35	Asp Gln Ala Ile Thr Pro Asp Gly Gln Gly Gly Gln Ile Gly Asp	
	115 120 125	
40	Asn Pro Leu Leu Lys Ala Met Leu Lys Leu Ile Ala Arg Met Met Asp	
	130 135 140	
	Gly Gln Ser Asp Gln Phe Gly Gln Pro Gly Thr Gly Asn Asn Ser Ala	
	145 150 155 160	
45	Ser Ser Gly Thr Ser Ser Ser Gly Gly Ser Pro Phe Asn Asp Leu Ser	
	165 170 175	
	Gly Gly Lys Ala Pro Ser Gly Asn Ser Pro Ser Gly Asn Tyr Ser Pro	
	180 185 190	
50	Val Ser Thr Phe Ser Pro Pro Ser Thr Pro Thr Ser Pro Thr Ser Pro	
	195 200 205	
55	Leu Asp Phe Pro Ser Ser Pro Thr Lys Ala Ala Gly Gly Ser Thr Pro	
	210 215 220	

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	Val	Thr	Asp	His	Pro	Asp	Pro	Val	Gly	Ser	Ala	Gly	Ile	Gly	Ala	Gly
	225							230			235			240		
5	Asn	Ser	Val	Ala	Phe	Thr	Ser	Ala	Gly	Ala	Asn	Gln	Thr	Val	Leu	His
					245				250				255			
10	Asp	Thr	Ile	Thr	Val	Lys	Ala	Gly	Gln	Val	Phe	Asp	Gly	Lys	Gly	Gln
					260			265				270				
15	Thr	Phe	Thr	Ala	Gly	Ser	Glu	Leu	Gly	Asp	Gly	Gly	Gln	Ser	Glu	Asn
					275			280			285					
20	Gln	Lys	Pro	Leu	Phe	Ile	Leu	Glu	Asp	Gly	Ala	Ser	Leu	Lys	Asn	Val
					290			295			300					
25	Thr	Met	Gly	Asp	Asp	Gly	Ala	Asp	Gly	Ile	His	Leu	Tyr	Gly	Asp	Ala
					305			310			315			320		
30	Lys	Ile	Asp	Asn	Leu	His	Val	Thr	Asn	Val	Gly	Glu	Asp	Ala	Ile	Thr
					325				330			335				
35	Val	Lys	Pro	Asn	Ser	Ala	Gly	Lys	Lys	Ser	His	Val	Glu	Ile	Thr	Asn
					340				345			350				
40	Ser	Ser	Phe	Glu	His	Ala	Ser	Asp	Lys	Ile	Leu	Gln	Leu	Asn	Ala	Asp
					355				360			365				
45	Thr	Asn	Leu	Ser	Val	Asp	Asn	Val	Lys	Ala	Lys	Asp	Phe	Gly	Thr	Phe
					370			375			380					
50	Val	Arg	Thr	Asn	Gly	Gly	Gln	Gln	Gly	Asn	Trp	Asp	Leu	Asn	Leu	Ser
					385			390			395			400		
55	His	Ile	Ser	Ala	Glu	Asp	Gly	Lys	Phe	Ser	Phe	Val	Lys	Ser	Asp	Ser
					405				410			415				
60	Glu	Gly	Leu	Asn	Val	Asn	Thr	Ser	Asp	Ile	Ser	Leu	Gly	Asp	Val	Glu
					420				425			430				
65	Asn	His	Tyr	Lys	Val	Pro	Met	Ser	Ala	Asn	Leu	Lys	Val	Ala	Glu	
					435				440			445				

This protein or polypeptide is acidic, rich in glycine and serine, and lacks cysteine. It is also heat stable, protease sensitive, and suppressed by inhibitors of plant metabolism. The protein or polypeptide of the present invention has a predicted molecular size of ca. 4.5 kDa.

Another potentially suitable hypersensitive response elicitor from *Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,663 which is hereby incorporated by reference. The protein is encoded by a DNA molecule having a nucleic acid sequence of SEQ. ID. No. 27 as follows:

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	ATGGAATTAA AATCACTGGG AACTAACAC AAGGCGGAG TACACACAGC GGCGCACAAAC	60
	CCTGTGGGC ATGGTGTG CTTACAGCAG GGCAGCAGCA GCAGCAGCCC GCAAAATGCC	120
5	GCTGCATCAT TGGCGGAGA AGGCAAAAT CGTGGAAAA TGCCGAGAAT TCACCAAGCA	180
	TCTACTGCAG CTGATGGTAT CAGCGCTGCT CACCAGAAA AGAAATCCTT CAGTCTCAGG	240
10	GGCTGTTGG GGACGAAAAA ATTTTCCAGA TCGGCACCGC AGGGCCAGCC AGGTACCAAC	300
	CACAGCAAAG GGGCAACATT GCGCGATCTG CTGGCGCGG ACGACGGCGA AACGCAGCAT	360
	GAGGCGGCCG CGCCAGATGC GGCGCGTTG ACCCGTTGG GCGGCGTCAA ACGCCGCAAT	420
15	ATGGACGACA TGGCCGGCG GCCAATGGTG AAAGGTGGCA GCGGCGAAGA TAAGGTACCA	480
	ACCGAGCAA AACGGCATCA GCTGAACAAAT TTTGGCCAGA TGCGCCAAAC GATGTTGAGC	540
20	AAAATGGCTC ACCCGGCTTC AGCCAACGCC GGCGATGCC TGCAGCATTG ACCGCCGCAC	600
	ATCCCGGGTA GCCACCACGA AATCAAGGAA GAACCGGTTG GCTCCACCAAG CAAGGCAACA	660
	ACGGCCCACG CAGACAGAGT GGAAATCGCT CAGGAAGATG ACGACAGCGA ATTCCAGCAA	720
25	CTGCATCAAC AGCGGCTGGC GCGCGAACGG GAAAATCCAC CGCAGCCGCC CAAACTCGGC	780
	GTTGCCACAC CGATTAGCGC CAGGTTTCAG CCCAAACTGA CTGCGGTTGC GGAAAGCGTC	840
	CTTGAGGGGA CAGATACCAAC GCAGTCACCC CTTAAGCCGC AATCAATGCT GAAAGGAAGT	900
30	GGAGCCGGGG TAACGCCGCT GGCGGTAACG CTGGATAAAG GCAAGTTGCA GCTGGCACCG	960
	GATAATCCAC CCGCGCTCAA TACGTTGTTG AAGCAGACAT TGGGAAAGA CACCCAGCAC	1020
35	TATCTGGCGC ACCATGCCAG CAGCGACGGT AGCCAGCATC TGCTGCTGGA CAACAAAGGC	1080
	CACCTGTTG ATATCAAAAG CACCGCCACC AGCTATAGCG TGCTGCACAA CAGCCACCCC	1140
40	GGTGAGATAA AGGGCAAGCT GGCGCAGGCG GGTACTGGCT CCGTCAGCGT AGACGGTAAA	1200
	AGCGGCAAGA TCTCGCTGGG GAGCGGTACG CAAAGTCACA ACAAAACAAT GCTAAGCCAA	1260
	CCGGGGGAAG CGCACCGTTC CTTATTAACC GGCATTTGGC AGCATCCTGC TGGCGCAGCG	1320
45	CGGCCGCAGG GCGAGTCAT CCGCCTGCAT GACGACAAAA TTCATATCCT GCATCCGGAG	1380
	CTGGCGTAT GGCAATCTGC GGATAAAAGAT ACCCACAGCC AGCTGTCTCG CCAGGCAGAC	1440
	GGTAAGCTCT ATGCGCTGAA AGACAACCGT ACCCTGCAAAC ACCTCTCCGA TAATAAAATCC	1500
50	TCAGAAAAGC TGGTCGATAA AATCAAATCG TATTCCGTTG ATCAGCGGGG GCAGGTGGCG	1560
	ATCCTGACGG ATACTCCCGG CCGCCATAAG ATGAGTATTA TGCCCTCGCT GGATGCTTCC	1620
55	CCGGAGAGCC ATATTCCT CAGCCTGCAT TTTGCCGATG CCCACCAAGGG GTTATTGCAC	1680
	GGGAAGTCGG AGCTTGAGGC ACAATCTGTC GCGATCAGCC ATGGCGACT GGTTGTGGCC	1740
60	GATAGCGAAG GCAAGCTGTT TAGGCCGCC ATTCCGAAGC AAGGGGATGG AAACGAACAG	1800
	AAAATGAAAG CCATGCCTCA GCATGCGCTC GATGAACATT TTGGTCATGA CCACCAGATT	1860
	TCTGGATTTC TCCATGACGA CCACGGCCAG CTTAATGCGC TGGTAAAAAA TAACTTCAGG	1920
65	CAGCAGCATG CCTGCCGTT GGGTAACGAT CATCAGTTTC ACCCCGGCTG GAACCTGACT	1980

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	GATGCGCTGG TTATCGACAA TCAGCTGGGG CTGCATCATA CCAATCCTGA ACCGCATGAG	2040
5	ATTCTTGATA TGGGGCATTG AGGCAGCCTG GCGTTACAGG AGGGCAAGCT TCACTATTTT	2100
	GACCAGCTGA CCAAAGGGTG GACTGGCGCG GAGTCAGATT GTAAGCAGCT GAAAAAAGGC	2160
	CTGGATGGAG CAGCTTATCT ACTGAAAGAC GGTGAAGTGA AACGCCGAA TATTAATCAG	2220
10	AGCACCTCCT CTATCAAGCA CGGAACGGAA AACGTTTTT CGCTGCCGCA TGTGCGCAAT	2280
	AAACCGGAGC CGGGAGATGC CCTGCAAGGG CTGAATAAG ACGATAAGGC CCAGGCCATG	2340
15	GCGGTGATTG GGGTAAATAA ATACCTGGCG CTGACGGAAA AAGGGACAT TCGCTCCTTC	2400
	CAGATAAAAC CCGGCACCCA GCAGTTGGAG CGGCCGGCAC AAACCTCTAG CCGCGAAGGT	2460
	ATCAGCGGCG AACTGAAAGA CATTGATGTC GACCACAAGC AGAACCTGTA TGCCCTTGACC	2520
20	CACGAGGGAG AGGTGTTCA TCAGCCGCGT GAAGCCTGGC AGAACCTGTC CGAAAGCAGC	2580
	AGCTGGCACA AACTGGCGTT GCCACAGAGT GAAAGTAAGC TAAAAAGTCT GGACATGAGC	2640
25	CATGAGCACA AACCGATTGC CACCTTGAA GACGGTAGCC AGCATCAGCT GAAGGCTGGC	2700
	GGCTGGCACG CCTATGCCGC ACCTGAACGC GGGCCGCTGG CGGTGGGTAC CAGCGGTTCA	2760
	CAAACCGTCT TTAACCGACT AATGCAGGGG GTGAAAGGCA AGGTGATCCC AGGCAGCGGG	2820
30	TTGACGGTTA AGCTCTCGGC TCAGACGGGG GGAATGACCG GCGCCGAAGG GCGCAAGGTC	2880
	AGCAGTAAAT TTTCCGAAAG GATCCGCGCC TATGCGTTCA ACCAACAAAT GTCCACGCCG	2940
35	CGACCGATTAA AAAATGCTGC TTATGCCACA CAGCACGGCT GGCAGGGCG TGAGGGTTG	3000
	AAGCCGTTGT ACGAGATGCA GGGAGCGCTG ATTAAACAAAC TGGATGCGCA TAACGTTCGT	3060
	CATAACGCGC CACAGCCAGA TTTGCAGAGC AAACCTGGAAA CTCTGGATT AGGCGAACAT	3120
40	GGCGCAGAAT TGCTTAACGA CATGAAGCGC TTCCCGACG AACTGGAGCA GAGTGCACC	3180
	CGITCGGTGA CCGTTTGTAG TCAACATCAG GGAGTGCTAA AAAGCAACGG TGAAATCAAT	3240
45	AGCGAATTAA AGCCATCGCC CGGCAAGGCG TTGGTCCAGA GCTTTAACGT CAATCGCTCT	3300
	GGTCAGGATC TAAGCAAGTC ACTGCAACAG GCAGTACATG CCACGCCGCC ATCCGCAGAG	3360
	AGTAAACTGC AATCCATGCT GGGGCACCTT GTCAGTGCAG GGGTGGATAT GAGTCATCAG	3420
50	AAGGGCGAGA TCCCGCTGGG CCGCCAGCGC GATCCGAATG ATAAAACCGC ACTGACCAAA	3480
	TCGCGTTTAA TTTTAGATAC CGTGACCATC GGTGAACCTGC ATGAACTGGC CGATAAGGCG	3540
55	AAACCTGGTAT CTGACCATAA ACCCGATGCC GATCAGATAA AACAGCTGCG CCAGCAGTTG	3600
	GATACGCTGC GTGAAAAGCG GTATGAGAGC AATCCGGTGA AGCATTACAC CGATATGGGC	3660
	TTCACCCATA ATAAGGCCTG GGAAGCAAAC TATGATGCCG TCAAAGCCTT TATCAATGCC	3720
60	TTTAAGAAAG AGCACCACGG CGTCAATCTG ACCACGCGTA CCGTACTGGC ATCACAGGGC	3780
	AGTGCAGGAGC TGGCGAAGAA GCTCAAGAAT ACGCTGTTGT CCCTGGACAG TGGTGAAGT	3840
65	ATGAGCTTCA GCCGGTCATA TGGCGGGGGC GTCAGCACTG TCTTTGTGCC TACCCCTAGC	3900

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	AAGAAGGTGC CAGTTCCGGT GATCCCCGGA GCCGGCATCA CGCTGGATCG CGCCTATAAC	3960
	CTGAGCTTCA GTCGTACCAAG CGGC GGATTG AACGTCAGTT TTGGCCGCGA CGGGCGGGTG	4020
5	AGTGGTAACA TCATGGTCGC TACCGGCCAT GATGTGATGC CCTATATGAC CGGTAAGAAA	4080
	ACCA GTACGCCAG TGACTGGTTG AGCGCAAAAC ATAAAATCAG CCCGGACTTG	4140
10	CGTATCGGCG CTGCTGTGAG TGGCACCCCTG CAAGGAACGC TACAAAACAG CCTGAAGTTT	4200
	AAGCTGACAG AGGATGAGCT GCCTGGCTTT ATCCATGGCT TGACGCATGG CACGTTGACC	4260
	CCGGCAGAAC TGTTGCAAAA GGGGATCGAA CATCAGATGA AGCAGGGCAG CAAACTGACG	4320
15	TTTAGCGTCG ATACCTCGGC AAATCTGGAT CTGCGTGCCG GTATCAATCT GAACGAAGAC	4380
	GGCAGTAAAC CAAATGGTGT CACTGCCGT GTTTCTGCCG GGCTAAGTGC ATCGGCAAAAC	4440
20	CTGGCCGCCG GCTCGCGTGA ACGCAGCACC ACCTCTGGCC AGTTTGGCAG CACGACTTCG	4500
	GCCAGCAATA ACCGCCAAC CTTCCCTAAC GGGGTCGGCG CGGGTGCTAA CCTGACGGCT	4560
	GCTTTAGGGG TTGCCCATTG ATCTACGCAT GAAGGGAAAC CGGTGGGAT CTTCCCGGCA	4620
25	TTTACCTCGA CCAATGTTTC GGCAGCGCTG GCGCTGGATA ACCGTACCTC ACAGAGTATC	4680
	AGCCTGGAAT TGAAGCGCGC GGAGCCGGTG ACCAGCAACG ATATCAGCGA GTTGACCTCC	4740
	ACGCTGGAA AACACTTAA GGATAGCGCC ACAACGAAGA TGCTTGCCGC TCTCAAAGAG	4800
30	TTAGATGACG CTAAGCCCGC TGAACAACTG CATATTTAC AGCAGCATTG CAGTGCAAAA	4860
	GATGTCGTCG GTGATGAACG CTACGAGGCG GTGCGCAACC TGAAAAAAACT GGTGATACGT	4920
35	CAACAGGCTG CGGACAGCCA CAGCATGGAA TTAGGATCTG CCAGTCACAG CACGACCTAC	4980
	AATAATCTGT CGAGAATAAA TAATGACGGC ATTGTCGAGC TGCTACACAA ACATTTCGAT	5040
40	GCAGCATTAC CAGCAAGCAG TGCCAAACGT CTTGGTGAAA TGATGAATAA CGATCCGGCA	5100
	CTGAAAGATA TTATTAAGCA GCTGCAAAGT ACGCCGTTCA GCAGCGCCAG CGTGTGATG	5160
	GAGCTGAAAG ATGGTCTGCG TGAGCAGACG GAAAAAGCAA TACTGGACGG TAAGGTGGT	5220
45	CGTGAAGAAG TGGGAGTACT TTTCCAGGAT CGTAACAACT TGCGTGTAA ATCGGTCAGC	5280
	GTCAGTCAGT CCGTCAGCAA AAGCGAAGGC TTCAATACCC CAGCGCTGTT ACTGGGGACG	5340
50	AGCAACAGCG CTGCTATGAG CATGGAGCGC AACATCGGAA CCATTAATTG TAAATACGGC	5400
	CAGGATCAGA ACACCCACG GCGATTTACC CTGGAGGGTG GAATAGCTCA GGCTAATCCG	5460
	CAGGTCGCAT CTGCGCTTAC TGATTGAAAG AAGGAAGGGC TGGAAATGAA GAGCTAA	5517

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This DNA molecule is known as the *dspE* gene for *Erwinia amylovora*. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ.

ID. No. 28 as follows:

- 17 -

	Met Glu Leu Lys Ser Leu Gly Thr Glu His Lys Ala Ala Val His Thr	
	1 5 10 15	
5	Ala Ala His Asn Pro Val Gly His Gly Val Ala Leu Gln Gln Gly Ser	
	20 25 30	
	Ser Ser Ser Ser Pro Gln Asn Ala Ala Ala Ser Leu Ala Ala Glu Gly	
	35 40 45	
10	Lys Asn Arg Gly Lys Met Pro Arg Ile His Gln Pro Ser Thr Ala Ala	
	50 55 60	
	Asp Gly Ile Ser Ala Ala His Gln Gln Lys Lys Ser Phe Ser Leu Arg	
	65 70 75 80	
15	Gly Cys Leu Gly Thr Lys Lys Phe Ser Arg Ser Ala Pro Gln Gly Gln	
	85 90 95	
20	Pro Gly Thr Thr His Ser Lys Gly Ala Thr Leu Arg Asp Leu Leu Ala	
	100 105 110	
	Arg Asp Asp Gly Glu Thr Gln His Glu Ala Ala Ala Pro Asp Ala Ala	
	115 120 125	
25	Arg Leu Thr Arg Ser Gly Gly Val Lys Arg Arg Asn Met Asp Asp Met	
	130 135 140	
	Ala Gly Arg Pro Met Val Lys Gly Gly Ser Gly Glu Asp Lys Val Pro	
	145 150 155 160	
30	Thr Gln Gln Lys Arg His Gln Leu Asn Asn Phe Gly Gln Met Arg Gln	
	165 170 175	
	Thr Met Leu Ser Lys Met Ala His Pro Ala Ser Ala Asn Ala Gly Asp	
35	180 185 190	
	Arg Leu Gln His Ser Pro Pro His Ile Pro Gly Ser His His Glu Ile	
	195 200 205	
40	Lys Glu Glu Pro Val Gly Ser Thr Ser Lys Ala Thr Thr Ala His Ala	
	210 215 220	
	Asp Arg Val Glu Ile Ala Gln Glu Asp Asp Asp Ser Glu Phe Gln Gln	
	225 230 235 240	
45	Leu His Gln Gln Arg Leu Ala Arg Glu Arg Glu Asn Pro Pro Gln Pro	
	245 250 255	
	Pro Lys Leu Gly Val Ala Thr Pro Ile Ser Ala Arg Phe Gln Pro Lys	
50	260 265 270	
	Leu Thr Ala Val Ala Glu Ser Val Leu Glu Gly Thr Asp Thr Thr Gln	
	275 280 285	
55	Ser Pro Leu Lys Pro Gln Ser Met Leu Lys Gly Ser Gly Ala Gly Val	
	290 295 300	
	Thr Pro Leu Ala Val Thr Leu Asp Lys Gly Lys Leu Gln Leu Ala Pro	
	305 310 315 320	
60	Asp Asn Pro Pro Ala Leu Asn Thr Leu Leu Lys Gln Thr Leu Gly Lys	
	325 330 335	
65	Asp Thr Gln His Tyr Leu Ala His His Ala Ser Ser Asp Gly Ser Gln	
	340 345 350	

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	His	Leu	Leu	Leu	Asp	Asn	Lys	Gly	His	Leu	Phe	Asp	Ile	Lys	Ser	Thr
	355						360						365			
5	Ala	Thr	Ser	Tyr	Ser	Val	Leu	His	Asn	Ser	His	Pro	Gly	Glu	Ile	Lys
	370					375						380				
	Gly	Lys	Leu	Ala	Gln	Ala	Gly	Thr	Gly	Ser	Val	Ser	Val	Asp	Gly	Lys
	385				390						395			400		
10	Ser	Gly	Lys	Ile	Ser	Leu	Gly	Ser	Gly	Thr	Gln	Ser	His	Asn	Lys	Thr
						405				410			415			
15	Met	Leu	Ser	Gln	Pro	Gly	Glu	Ala	His	Arg	Ser	Leu	Leu	Thr	Gly	Ile
					420					425			430			
	Trp	Gln	His	Pro	Ala	Gly	Ala	Ala	Arg	Pro	Gln	Gly	Glu	Ser	Ile	Arg
					435					440			445			
20	Leu	His	Asp	Asp	Lys	Ile	His	Ile	Leu	His	Pro	Glu	Leu	Gly	Val	Trp
					450					455			460			
	Gln	Ser	Ala	Asp	Lys	Asp	Thr	His	Ser	Gln	Leu	Ser	Arg	Gln	Ala	Asp
						470					475			480		
25	Gly	Lys	Leu	Tyr	Ala	Leu	Lys	Asp	Asn	Arg	Thr	Leu	Gln	Asn	Leu	Ser
						485					490			495		
	Asp	Asn	Lys	Ser	Ser	Glu	Lys	Leu	Val	Asp	Lys	Ile	Lys	Ser	Tyr	Ser
						500					505			510		
30	Val	Asp	Gln	Arg	Gly	Gln	Val	Ala	Ile	Leu	Thr	Asp	Thr	Pro	Gly	Arg
						515					520			525		
35	His	Lys	Met	Ser	Ile	Met	Pro	Ser	Leu	Asp	Ala	Ser	Pro	Glu	Ser	His
						530					535			540		
	Ile	Ser	Leu	Ser	Leu	His	Phe	Ala	Asp	Ala	His	Gln	Gly	Leu	Leu	His
						545					550			555		560
40	Gly	Lys	Ser	Glu	Leu	Glu	Ala	Gln	Ser	Val	Ala	Ile	Ser	His	Gly	Arg
						565					570			575		
	Leu	Val	Val	Ala	Asp	Ser	Glu	Gly	Lys	Leu	Phe	Ser	Ala	Ala	Ile	Pro
						580					585			590		
45	Lys	Gln	Gly	Asp	Gly	Asn	Glu	Leu	Lys	Met	Lys	Ala	Met	Pro	Gln	His
						595					600			605		
	Ala	Leu	Asp	Glu	His	Phe	Gly	His	Asp	His	Gln	Ile	Ser	Gly	Phe	Phe
						610					615			620		
50	His	Asp	Asp	His	Gly	Gln	Leu	Asn	Ala	Leu	Val	Lys	Asn	Asn	Phe	Arg
						625					630			635		640
	Gln	Gln	His	Ala	Cys	Pro	Leu	Gly	Asn	Asp	His	Gln	Phe	His	Pro	Gly
							645					650			655	
55	Trp	Asn	Leu	Thr	Asp	Ala	Leu	Val	Ile	Asp	Asn	Gln	Leu	Gly	Leu	His
							660					665			670	
	His	Thr	Asn	Pro	Glu	Pro	His	Glu	Ile	Leu	Asp	Met	Gly	His	Leu	Gly
							675					680			685	

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Ser Leu Ala Leu Gln Glu Gly Lys Leu His Tyr Phe Asp Gln Leu Thr
 690 695 700
 5 Lys Gly Trp Thr Gly Ala Glu Ser Asp Cys Lys Gln Leu Lys Lys Gly
 705 710 715 720
 Leu Asp Gly Ala Ala Tyr Leu Leu Lys Asp Gly Glu Val Lys Arg Leu
 725 730 735
 10 Asn Ile Asn Gln Ser Thr Ser Ile Lys His Gly Thr Glu Asn Val
 740 745 750
 Phe Ser Leu Pro His Val Arg Asn Lys Pro Glu Pro Gly Asp Ala Leu
 755 760 765
 15 Gln Gly Leu Asn Lys Asp Asp Lys Ala Gln Ala Met Ala Val Ile Gly
 770 775 780
 20 Val Asn Lys Tyr Leu Ala Leu Thr Glu Lys Gly Asp Ile Arg Ser Phe
 785 790 795 800
 Gln Ile Lys Pro Gly Thr Gln Gln Leu Glu Arg Pro Ala Gln Thr Leu
 805 810 815
 25 Ser Arg Glu Gly Ile Ser Gly Glu Leu Lys Asp Ile His Val Asp His
 820 825 830
 Lys Gln Asn Leu Tyr Ala Leu Thr His Glu Gly Glu Val Phe His Gln
 835 840 845
 30 Pro Arg Glu Ala Trp Gln Asn Gly Ala Glu Ser Ser Ser Trp His Lys
 850 855 860
 Leu Ala Leu Pro Gln Ser Glu Ser Lys Leu Lys Ser Leu Asp Met Ser
 35 865 870 875 880
 His Glu His Lys Pro Ile Ala Thr Phe Glu Asp Gly Ser Gln His Gln
 885 890 895
 40 Leu Lys Ala Gly Gly Trp His Ala Tyr Ala Ala Pro Glu Arg Gly Pro
 900 905 910
 Leu Ala Val Gly Thr Ser Gly Ser Gln Thr Val Phe Asn Arg Leu Met
 45 915 920 925
 Gln Gly Val Lys Gly Lys Val Ile Pro Gly Ser Gly Leu Thr Val Lys
 930 935 940
 50 Leu Ser Ala Gln Thr Gly Gly Met Thr Gly Ala Glu Gly Arg Lys Val
 945 950 955 960
 Ser Ser Lys Phe Ser Glu Arg Ile Arg Ala Tyr Ala Phe Asn Pro Thr
 965 970 975
 55 Met Ser Thr Pro Arg Pro Ile Lys Asn Ala Ala Tyr Ala Thr Gln His
 980 985 990
 Gly Trp Gln Gly Arg Glu Gly Leu Lys Pro Leu Tyr Glu Met Gln Gly
 60 995 1000 1005
 Ala Leu Ile Lys Gln Leu Asp Ala His Asn Val Arg His Asn Ala Pro
 1010 1015 1020
 65 Gln Pro Asp Leu Gln Ser Lys Leu Glu Thr Leu Asp Leu Gly Glu His
 1025 1030 1035 1040

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Gly Ala Glu Leu Leu Asn Asp Met Lys Arg Phe Arg Asp Glu Leu Glu
 1045 1050 1055

5 Gln Ser Ala Thr Arg Ser Val Thr Val Leu Gly Gln His Gln Gly Val
 1060 1065 1070

Leu Lys Ser Asn Gly Glu Ile Asn Ser Glu Phe Lys Pro Ser Pro Gly
 1075 1080 1085

10 Lys Ala Leu Val Gln Ser Phe Asn Val Asn Arg Ser Gly Gln Asp Leu
 1090 1095 1100

15 Ser Lys Ser Leu Gln Gln Ala Val His Ala Thr Pro Pro Ser Ala Glu
 1105 1110 1115 1120

Ser Lys Leu Gln Ser Met Leu Gly His Phe Val Ser Ala Gly Val Asp
 1125 1130 1135

20 Met Ser His Gln Lys Gly Glu Ile Pro Leu Gly Arg Gln Arg Asp Pro
 1140 1145 1150

Asn Asp Lys Thr Ala Leu Thr Lys Ser Arg Leu Ile Leu Asp Thr Val
 1155 1160 1165

25 Thr Ile Gly Glu Leu His Glu Leu Ala Asp Lys Ala Lys Leu Val Ser
 1170 1175 1180

30 Asp His Lys Pro Asp Ala Asp Gln Ile Lys Gln Leu Arg Gln Gln Phe
 1185 1190 1195 1200

Asp Thr Leu Arg Glu Lys Arg Tyr Glu Ser Asn Pro Val Lys His Tyr
 1205 1210 1215

35 Thr Asp Met Gly Phe Thr His Asn Lys Ala Leu Glu Ala Asn Tyr Asp
 1220 1225 1230

Ala Val Lys Ala Phe Ile Asn Ala Phe Lys Lys Glu His His Gly Val
 1235 1240 1245

40 Asn Leu Thr Thr Arg Thr Val Leu Glu Ser Gln Gly Ser Ala Glu Leu
 1250 1255 1260

45 Ala Lys Lys Leu Lys Asn Thr Leu Leu Ser Leu Asp Ser Gly Glu Ser
 1265 1270 1275 1280

Met Ser Phe Ser Arg Ser Tyr Gly Gly Val Ser Thr Val Phe Val
 1285 1290 1295

50 Pro Thr Leu Ser Lys Lys Val Pro Val Pro Val Ile Pro Gly Ala Gly
 1300 1305 1310

Ile Thr Leu Asp Arg Ala Tyr Asn Leu Ser Phe Ser Arg Thr Ser Gly
 1315 1320 1325

55 Gly Leu Asn Val Ser Phe Gly Arg Asp Gly Gly Val Ser Gly Asn Ile
 1330 1335 1340

60 Met Val Ala Thr Gly His Asp Val Met Pro Tyr Met Thr Gly Lys Lys
 1345 1350 1355 1360

Thr Ser Ala Gly Asn Ala Ser Asp Trp Leu Ser Ala Lys His Lys Ile
 1365 1370 1375

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Ser Pro Asp Leu Arg Ile Gly Ala Ala Val Ser Gly Thr Leu Gln Gly
 1380 1385 1390

 5 Thr Leu Gln Asn Ser Leu Lys Phe Lys Leu Thr Glu Asp Glu Leu Pro
 1395 1400 1405

 Gly Phe Ile His Gly Leu Thr His Gly Thr Leu Thr Pro Ala Glu Leu
 1410 1415 1420

 10 Leu Gln Lys Gly Ile Glu His Gln Met Lys Gln Gly Ser Lys Leu Thr
 1425 1430 1435 1440

 Phe Ser Val Asp Thr Ser Ala Asn Leu Asp Leu Arg Ala Gly Ile Asn
 1445 1450 1455

 15 Leu Asn Glu Asp Gly Ser Lys Pro Asn Gly Val Thr Ala Arg Val Ser
 1460 1465 1470

 Ala Gly Leu Ser Ala Ser Ala Asn Leu Ala Ala Gly Ser Arg Glu Arg
 20 1475 1480 1485

 Ser Thr Thr Ser Gly Gln Phe Gly Ser Thr Thr Ser Ala Ser Asn Asn
 1490 1495 1500

 25 Arg Pro Thr Phe Leu Asn Gly Val Gly Ala Gly Ala Asn Leu Thr Ala
 1505 1510 1515 1520

 Ala Leu Gly Val Ala His Ser Ser Thr His Glu Gly Lys Pro Val Gly
 1525 1530 1535

 30 Ile Phe Pro Ala Phe Thr Ser Thr Asn Val Ser Ala Ala Leu Ala Leu
 1540 1545 1550

 Asp Asn Arg Thr Ser Gln Ser Ile Ser Leu Glu Leu Lys Arg Ala Glu
 35 1555 1560 1565

 Pro Val Thr Ser Asn Asp Ile Ser Glu Leu Thr Ser Thr Leu Gly Lys
 1570 1575 1580

 40 His Phe Lys Asp Ser Ala Thr Thr Lys Met Leu Ala Ala Leu Lys Glu
 1585 1590 1595 1600

 Leu Asp Asp Ala Lys Pro Ala Glu Gln Leu His Ile Leu Gln Gln His
 45 1605 1610 1615

 Phe Ser Ala Lys Asp Val Val Gly Asp Glu Arg Tyr Glu Ala Val Arg
 1620 1625 1630

 50 Asn Leu Lys Lys Leu Val Ile Arg Gln Gln Ala Ala Asp Ser His Ser
 1635 1640 1645

 Met Glu Leu Gly Ser Ala Ser His Ser Thr Thr Tyr Asn Asn Leu Ser
 1650 1655 1660

 55 Arg Ile Asn Asn Asp Gly Ile Val Glu Leu Leu His Lys His Phe Asp
 1665 1670 1675 1680

 Ala Ala Leu Pro Ala Ser Ser Ala Lys Arg Leu Gly Glu Met Met Asn
 60 1685 1690 1695

 Asn Asp Pro Ala Leu Lys Asp Ile Ile Lys Gln Leu Gln Ser Thr Pro
 1700 1705 1710

 65 Phe Ser Ser Ala Ser Val Ser Met Glu Leu Lys Asp Gly Leu Arg Glu
 1715 1720 1725

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	Gln	Thr	Glu	Lys	Ala	Ile	Leu	Asp	Gly	Lys	Val	Gly	Arg	Glu	Glu	Val
	1730						1735					1740				
5	Gly	Val	Leu	Phe	Gln	Asp	Arg	Asn	Asn	Leu	Arg	Val	Lys	Ser	Val	Ser
	1745						1750				1755					1760
	Val	Ser	Gln	Ser	Val	Ser	Lys	Ser	Glu	Gly	Phe	Asn	Thr	Pro	Ala	Leu
											1770					1775
10	Leu	Leu	Gly	Thr	Ser	Asn	Ser	Ala	Ala	Met	Ser	Met	Glu	Arg	Asn	Ile
									1780		1785		1790			
	Gly	Thr	Ile	Asn	Phe	Lys	Tyr	Gly	Gln	Asp	Gln	Asn	Thr	Pro	Arg	Arg
15									1795		1800		1805			
	Phe	Thr	Leu	Glu	Gly	Gly	Ile	Ala	Gln	Ala	Asn	Pro	Gln	Val	Ala	Ser
									1810		1815		1820			
20	Ala	Leu	Thr	Asp	Leu	Lys	Lys	Glu	Gly	Leu	Glu	Met	Lys	Ser		
								1825		1830		1835				

This protein or polypeptide is about 198 kDa and has a pI of 8.98.

25 The present invention relates to an isolated DNA molecule having a nucleotide sequence of SEQ. ID. No. 29 as follows:

30	ATGACATCGT CACAGCAGCG GGTTGAAAGG TTTTTACAGT ATTTCTCCGC CGGGTGTAAA ACGCCCATAC ATCTGAAAGA CGGGGTGTGC GCCCTGTATA ACGAACAAAGA TGAGGAGGCG GCGGTGCTGG AAGTACCGCA ACACAGCGAC AGCCTGTTAC TACACTGCCG AATCATTGAG	60 120 180
35	GCTGACCCAC AAACCTCAAT AACCCGTAT TCGATGCTAT TACAGCTGAA TTTTGAAATG GCGGCCATGC GCGGCTGTTG GCTGGCGCTG GATGAACTGC ACAACGTGCG TTTATGTTT CAGCAGTCGC TGGAGCATCT GGATGAAGCA AGTTTTAGCG ATATCGTTAG CGGCTTCATC	240 300 360
40	GAACATGCGG CAGAACTGCG TGAGTATATA GCGCAATTAG ACGAGAGTAG CGCGGCATAA	420

This is known as the *dspF* gene. This isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 30 as follows:

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	Thr	Ser	Ile	Thr	Leu	Tyr	Ser	Met	Leu	Leu	Gln	Leu	Asn	Phe	Glu	Met
	65					70					75				80	
5	Ala	Ala	Met	Arg	Gly	Cys	Trp	Leu	Ala	Leu	Asp	Glu	Leu	His	Asn	Val
					85					90				95		
10	Arg	Leu	Cys	Phe	Gln	Gln	Ser	Leu	Glu	His	Leu	Asp	Glu	Ala	Ser	Phe
					100				105				110			
15	Ser	Asp	Ile	Val	Ser	Gly	Phe	Ile	Glu	His	Ala	Ala	Glu	Val	Arg	Glu
					115				120				125			
	Tyr	Ile	Ala	Gln	Leu	Asp	Glu	Ser	Ser	Ala	Ala					
					130				135							

This protein or polypeptide is about 16 kDa and has a pI of 4.45.

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID.

20 No. 31 as follows:

	Met	Gln	Ser	Leu	Ser	Leu	Asn	Ser	Ser	Ser	Leu	Gln	Thr	Pro	Ala	Met
	1				5						10				15	
25	Ala	Leu	Val	Leu	Val	Arg	Pro	Glu	Ala	Glu	Thr	Thr	Gly	Ser	Thr	Ser
					20					25				30		
30	Ser	Lys	Ala	Leu	Gln	Glu	Val	Val	Val	Lys	Leu	Ala	Glu	Glu	Leu	Met
					35				40				45			
35	Arg	Asn	Gly	Gln	Leu	Asp	Asp	Ser	Ser	Pro	Leu	Gly	Lys	Leu	Leu	Ala
					50				55				60			
40	Lys	Ser	Met	Ala	Ala	Asp	Gly	Lys	Ala	Gly	Gly	Gly	Ile	Glu	Asp	Val
					65				70				75		80	
	Ile	Ala	Ala	Leu	Asp	Lys	Leu	Ile	His	Glu	Lys	Leu	Gly	Asp	Asn	Phe
					85					90				95		
45	Gly	Ala	Ser	Ala	Asp	Ser	Ala	Ser	Gly	Thr	Gly	Gln	Gln	Asp	Leu	Met
					100				105				110			
50	Thr	Gln	Val	Leu	Asn	Gly	Leu	Ala	Lys	Ser	Met	Leu	Asp	Asp	Leu	Leu
					115				120				125			
55	Thr	Lys	Gln	Asp	Gly	Gly	Thr	Ser	Phe	Ser	Glu	Asp	Asp	Met	Pro	Met
					130				135				140			
60	Leu	Asn	Lys	Ile	Ala	Gln	Phe	Met	Asp	Asp	Asn	Pro	Ala	Gln	Phe	Pro
					145				150				155		160	
65	Lys	Pro	Asp	Ser	Gly	Ser	Trp	Val	Asn	Glu	Leu	Lys	Glu	Asp	Asn	Phe
					165				170				175			
70	Leu	Asp	Gly	Asp	Glu	Thr	Ala	Ala	Phe	Arg	Ser	Ala	Leu	Asp	Ile	Ile
					180				185				190			

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	Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly			
	195	200	205	
	Thr Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser			
	210	215	220	
5	Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser			
	225	230	235	240
	Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp			
	245	250	255	
10	Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Leu Gly Thr Pro Val			
	260	265	270	
	Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln			
	275	280	285	
	Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Lys Gly Leu Glu Ala			
	290	295	300	
15	Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala			
	305	310	315	320
	Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg			
	325	330	335	
20	Asn Gln Ala Ala Ala			
	340			

This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine.

25 Further information about the hypersensitive response elicitor derived from *Pseudomonas syringae* is found in He, S. Y., H. C. Huang, and A. Collmer, "Pseudomonas syringae pv. syringae Harpin_{Ps}: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," *Cell* 73:1255-1266 (1993), which is hereby incorporated by reference. The DNA molecule encoding the 30 hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 32 as follows:

	ATGCAGAGTC TCAGTCTTAA CAGCAGCTCG CTGCAAACCC CGGCAATGGC CCTTGTCCCTG	60
	GTACGTCCTG AAGCCGAGAC GACTGGCAGT ACGTCGAGCA AGGCGCTTCA GGAAGTTGTC	120
35	GTGAAGCTGG CCGAGGAACT GATGCGCAAT GGTCAACTCG ACGACAGCTC GCCATTGGGA	180
	AAACTGTTGG CCAAGTCGAT GGCCGCAGAT GGCAAGGCGG GCGGCGGTAT TGAGGATGTC	240
	ATCGCTGCGC TGGACAAGCT GATCCATGAA AAGCTCGGTG ACAACTTCGG CGCGTCTGCG	300

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	GACAGCGCCT	CGGGTACCGG	ACAGCAGGAC	CTGATGACTC	AGGTGCTCAA	TGGCCTGGCC	360
	AAGTCGATGC	TCGATGATCT	TCTGACCAAG	CAGGATGGCG	GGACAAGCTT	CTCCGAAGAC	420
	GATATGCCGA	TGCTGAACAA	GATCGCGCAG	TTCATGGATG	ACAATCCCGC	ACAGTTTCCC	480
	AAGCCGGACT	CGGGCTCCTG	GGTGAACGAA	CTCAAGGAAG	ACAACCTCCT	TGATGGCGAC	540
5	GAAACGGCTG	CGTTCCGTTTC	GGCACTCGAC	ATCATTGCC	AGCAACTGGG	TAATCAGCAG	600
	AGTGACGCTG	GCAGTCTGGC	AGGGACGGGT	GGAGGTCTGG	GCACTCCGAG	CAGTTTTCC	660
	AACAACCTCGT	CCGTGATGGG	TGATCCGCTG	ATCGACGCCA	ATACCGTCC	CGGTGACAGC	720
	GGCAATAACCC	GTGGTGAAGC	GGGGCAACTG	ATCGGCGAGC	TTATCGACCG	TGGCCTGCAA	780
	TCGGTATTGG	CCGGTGGTGG	ACTGGGCACA	CCCGTAAACA	CCCCGCAGAC	CGGTACGTCG	840
10	GCGAATGGCG	GACAGTCCGC	TCAGGATCTT	GATCAGTTGC	TGGGCGGCTT	GCTGCTCAAG	900
	CGCGTGGAGG	CAACGGCTAA	GGATGCCGGG	CAAACAGGCA	CCGACGTGCA	GTCGAGCGCT	960
	GCGCAAATCG	CCACCTTGCT	GGTCAGTACG	CTGCTGCAAG	GCACCCGCAA	TCAGGCTGCA	1020
	GCCTGA						1026

15 Another potentially suitable hypersensitive response elicitor from *Pseudomonas syringae* is disclosed in U.S. Patent Application Serial No. 09/120,817, which is hereby incorporated by reference. The protein has a nucleotide sequence of SEQ. ID. No. 33 as follows:

20	TCCACTTCGC TGATTTGAA ATTGGCAGAT TCATAGAAC GTTCAGGTGT GGAAATCAGG CTGAGTGCAG AGATTCGTT GATAAGGGTG TGGTACTGGT CATTGTTGGT CATTTCAGG	60 120
25	CCTCTGAGTG CGGTGCGGAG CAATACCAAGT CTTCCCTGCTG GCGTGTGCAC ACTGAGTCGC AGGCATAGGC ATTTCAGTTC CTTGCCTGGG TTGGGCATAT AAAAAAAAGGA ACTTTTAAAAA	180 240
30	ACAGTGCAAT GAGATGCCGG CAAAACGGGA ACCGGTCGCT GCGCTTGCC ACTCACTTCG AGCAAGCTCA ACCCCAAACA TCCACATCCC TATCGAACGG ACAGCGATAC GGCCACTTGC	300 360
35	TCTGGTAAAC CCTGGAGCTG GCGTCGGTCC AATTGCCAC TTAGCGAGGT AACGCAGCAT GAGCATCGGC ATCACACCCCC GGCCGCAACA GACCACCACG CCACTCGATT TTTGGCGCT	420 480
40	AAGCGGCAAG AGTCCTCAAC CAAACACGTT CGGCGAGCAG AACACTCAGC AAGCGATCGA CCCGAGTGCA CTGTTGTTCG GCAGCGACAC ACAGAAAGAC GTCAACTTCG GCACGCCGA CAGCACCGTC CAGAATCCGC AGGACGCCAG CAAGCCCAAC GACAGCCAGT CCAACATCGC	540 600 660
	TAAAATTGATC AGTGCATTGA TCATGTCGTT GCTGCAGATG CTCACCAACT CCAATAAAA GCAGGACACC AATCAGGAAC AGCCTGATAG CCAGGCTCCT TTCCAGAACACACCGGGCT	720 780

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CGGTACACCG	TCGGCCGATA	GCGGGGCGG	CGGTACACCG	GATGCGACAG	GTGGCGGCGG	840	
5	CGGTGATACT	CCAAGCGCAA	CAGGCCGTGG	CGGCCGGTGT	ACTCCGACCG	CAACAGGCCG	900
TGGCGGCAGC	GGTGGCGGCG	GCACACCCAC	TGCAACAGGT	GGCGGCAGCG	GTGGCACACC	960	
10	CACTGCAACA	GGCGGTGGCG	AGGGTGGCGT	AACACCGCAA	ATCACTCCGC	AGTTGGCCAA	1020
CCCTAACCGT	ACCTCAGGTA	CTGGCTCGGT	GTGGGACACC	GCAGGTTCTA	CCGAGCAAGC	1080	
15	CGGCAAGATC	AATGTGGTGA	AAGACACCAT	CAAGGTCGGC	GCTGGCGAAG	TCTTTGACGG	1140
CCACGGCGCA	ACCTTCACTG	CCGACAAATC	TATGGGTAAC	GGAGACCAGG	GCGAAAATCA	1200	
GAAGCCCAGT	TTCGAGCTGG	CTGAAGGCGC	TACGTTGAAG	AATGTGAACC	TGGGTGAGAA	1260	
20	CGAGGTCGAT	GGCATCCACG	TGAAAGCCAA	AAACGCTCAG	GAAGTCACCA	TTGACAACGT	1320
GCATGCCAG	AACGTCGGTG	AAGACCTGAT	TACGGTCAAA	GGCGAGGGAG	GCGCAGCGGT	1380	
25	CACTAATCTG	AACATCAAGA	ACAGCAGTGC	CAAAGGTGCA	GACGACAAGG	TTGTCCAGCT	1440
CAACGCCAAC	ACTCACTTGA	AAATCGACAA	CTTCAAGGCC	GACGATTTCG	GCACGATGGT	1500	
30	TCGCACCAAC	GGTGGCAAGC	AGTTTGATGA	CATGAGCATC	GAGCTGAACG	GCATCGAAGC	1560
TAACCACGGC	AAGTTCGCCC	TGGTAAAAG	CGACAGTGAC	GATCTGAAGC	TGGCAACGGG	1620	
CAACATCGCC	ATGACCGACG	TCAAACACGC	CTACGATAAA	ACCCAGGCAT	CGACCCAACA	1680	
CACCGAGCTT	TGAATCCAGA	CAAGTAGCTT	GAAAAAAGGG	GGTGGACTC		1729	

35 This DNA molecule is known as the *dspE* gene for *Pseudomonas syringae*. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ. ID. No. 34 as follows:

40	Met	Ser	Ile	Gly	Ile	Thr	Pro	Arg	Pro	Gln	Gln	Thr	Thr	Thr	Pro	Leu
	1				5					10				15		
	Asp	Phe	Ser	Ala	Leu	Ser	Gly	Lys	Ser	Pro	Gln	Pro	Asn	Thr	Phe	Gly
45		20				25			25				30			
	Glu	Gln	Asn	Thr	Gln	Gln	Ala	Ile	Asp	Pro	Ser	Ala	Leu	Leu	Phe	Gly
		35					40						45			
50	Ser	Asp	Thr	Gln	Lys	Asp	Val	Asn	Phe	Gly	Thr	Pro	Asp	Ser	Thr	Val
		50				55			55		60					
	Gln	Asn	Pro	Gln	Asp	Ala	Ser	Lys	Pro	Asn	Asp	Ser	Gln	Ser	Asn	Ile
55		65			70			70		75			80			
	Ala	Lys	Leu	Ile	Ser	Ala	Leu	Ile	Met	Ser	Leu	Leu	Gln	Met	Leu	Thr
					85				90				95			

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	Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln			
	100	105	110	
5	Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser			
	115	120	125	
	Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Asp Thr			
	130	135	140	
10	Pro Ser Ala Thr Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly			
	145	150	155	160
	Gly Gly Gly Ser Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly			
	165	170	175	
15	Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Glu Gly Val Thr			
	180	185	190	
20	Pro Gln Ile Thr Pro Gln Leu Ala Asn Pro Asn Arg Thr Ser Gly Thr			
	195	200	205	
	Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile			
	210	215	220	
25	Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp			
	225	230	235	240
	Gly His Gly Ala Thr Phe Thr Ala Asp Lys Ser Met Gly Asn Gly Asp			
	245	250	255	
30	Gln Gly Glu Asn Gln Lys Pro Met Phe Glu Leu Ala Glu Gly Ala Thr			
	260	265	270	
35	Leu Lys Asn Val Asn Leu Gly Glu Asn Glu Val Asp Gly Ile His Val			
	275	280	285	
	Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln			
	290	295	300	
40	Asn Val Gly Glu Asp Leu Ile Thr Val Lys Gly Glu Gly Ala Ala			
	305	310	315	320
	Val Thr Asn Leu Asn Ile Lys Asn Ser Ser Ala Lys Gly Ala Asp Asp			
	325	330	335	
45	Lys Val Val Gln Leu Asn Ala Asn Thr His Leu Lys Ile Asp Asn Phe			
	340	345	350	
50	Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln			
	355	360	365	
	Phe Asp Asp Met Ser Ile Glu Leu Asn Gly Ile Glu Ala Asn His Gly			
	370	375	380	
55	Lys Phe Ala Leu Val Lys Ser Asp Ser Asp Asp Leu Lys Leu Ala Thr			
	385	390	395	400
	Gly Asn Ile Ala Met Thr Asp Val Lys His Ala Tyr Asp Lys Thr Gln			
	405	410	415	

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Ala Ser Thr Gln His Thr Glu Leu
420

5

This protein or polypeptide is about 42.9 kDa.

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ.

10 ID. No. 35 as follows:

	Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln
	1 5 10 15
15	Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser
	20 25 30
	Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile
	35 40 45
	Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly
	50 55 60
20	Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala
	65 70 75 80
	Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser
	85 90 95
25	Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met
	100 105 110
	Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala
	115 120 125
	Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val
	130 135 140
30	Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala
	145 150 155 160
	Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly
	165 170 175
35	Gly Ala Gly Ala Gly Gly Ala Gly Gly Val Gly Gly Ala Gly Gly
	180 185 190
	Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala
	195 200 205
	Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn
	210 215 220

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	Ala	Gly	Asp	Val	Asn	Gly	Ala	Asn	Gly	Ala	Asp	Asp	Gly	Ser	Glu	Asp
	225				230				235				240			
	Gln	Gly	Gly	Leu	Thr	Gly	Val	Leu	Gln	Lys	Leu	Met	Lys	Ile	Leu	Asn
					245				250				255			
5	Ala	Leu	Val	Gln	Met	Met	Gln	Gln	Gly	Gly	Leu	Gly	Gly	Asn	Gln	
					260				265				270			
	Ala	Gln	Gly	Gly	Ser	Lys	Gly	Ala	Gly	Asn	Ala	Ser	Pro	Ala	Ser	Gly
					275				280				285			
10	Ala	Asn	Pro	Gly	Ala	Asn	Gln	Pro	Gly	Ser	Ala	Asp	Asp	Gln	Ser	Ser
					290				295				300			
	Gly	Gln	Asn	Asn	Leu	Gln	Ser	Gln	Ile	Met	Asp	Val	Val	Lys	Glu	Val
					305				310				315			320
	Val	Gln	Ile	Leu	Gln	Gln	Met	Leu	Ala	Ala	Gln	Asn	Gly	Gly	Ser	Gln
							325				330				335	
15	Gln	Ser	Thr	Ser	Thr	Gln	Pro	Met								
					340											

It is encoded by a DNA molecule having a nucleotide sequence corresponding SEQ.

ID. No. 36 as follows:

	ATGTCAGTCG	GAAACATCCA	GAGCCCGTCG	AACCTCCCGG	GTCTGCAGAA	CCTGAACCTC	60
20	AACACCAACA	CCAACAGCCA	GCAATCGGGC	CAGTCCGTGC	AAGACCTGAT	CAAGCAGGTC	120
	GAGAAGGACA	TCCTCAACAT	CATCGCAGCC	CTCGTGCAGA	AGGCCGCACA	GTCGGCGGGC	180
	GGCAACACCG	GTAACACCGG	CAACCGGCCG	GCGAAGGACG	GCAATGCCAA	CGCGGGCGCC	240
	AACGACCCGA	GCAAGAACGA	CCCGAGCAAG	AGCCAGGCTC	CGCAGTCGGC	CAACAAGACC	300
	GGCAACGTCG	ACGACGCCAA	CAACCAGGAT	CCGATGCAAG	CGCTGATGCA	GCTGCTGGAA	360
25	GACCTGGTGA	AGCTGCTGAA	GGCGGCCCTG	CACATGCAGC	AGCCCGGCGG	CAATGACAAG	420
	GGCAACGGCG	TGGCGGTGC	CAACGGCGCC	AAGGGTGCCG	GCGGCCAGGG	CGGCCTGGCC	480
	GAAGCGCTGC	AGGAGATCGA	GCAGATCCTC	GCCCAGCTCG	GCGGCGGCGG	TGCTGGCGCC	540
	GGCGCGCGG	GTGGCGGTGT	CGGCGGTGCT	GGTGGCGCGG	ATGGCGGCTC	CGGTGCGGGT	600
	GGCGCAGGCG	GTGCGAACGG	CGCCGACGGC	GGCAATGGCG	TGAACGGCAA	CCAGGCGAAC	660
30	GGCCCGCAGA	ACGCAGGCAG	TGTCAACGGT	GCCAACGGCG	CGGATGACGG	CAGCGAACAC	720
	CAGGGCGGCC	TCACCGGCGT	GCTGAAAAG	CTGATGAAGA	TCCTGAACGC	GCTGGTGCAG	780
	ATGATGCAGC	AAGGCGGCC	CGGCGGCC	AACCAGGCGC	AGGGCGGCTC	GAAGGGTGCC	840
	GGCAACGCCT	CGCCGGCTTC	CGGCGCGAAC	CCGGGCGCGA	ACCAGCCCGG	TTCGGCGGGAT	900

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GATCAATCGT CGGCCAGAA CAATCTGCAA TCCCAGATCA TGGATGTGGT GAAGGAGGTC 960
GTCCAGATCC TGCAGCGAGAT GCTGGCGGCG CAGAACGGCG GCAGGCCAGCA GTCCACCTCG 1020
ACGCAGCCGA TGTAA 1035

5

Further information regarding the hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* is set forth in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994), which is hereby incorporated by reference.

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. *glycines* has an amino acid sequence corresponding to SEQ. ID. No. 37 as follows:

15

Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala
 1 5 10 15
 Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr
 20 25

20

This sequence is an amino terminal sequence having only 26 residues from the hypersensitive response elicitor polypeptide or protein of *Xanthomonas campestris* pv. glycines. It matches with fimbrial subunit proteins determined in other 25 *Xanthomonas campestris* pathovars.

25 *Xanthomonas campestris* pathovars.

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. *pelargonii* is heat stable, protease sensitive, and has a molecular weight of 20 kDa. It includes an amino acid sequence corresponding to SEQ. ID. No. 38 as follows:

30

Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln
1 5 10 15
Leu Leu Ala Met
20

35

Isolation of *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui et al., "The RsmA Mutants of *Erwinia carotovora*

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subsp. *carotovora* Strain Ecc71 Overexpress *hrp N_{Ecc}* and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference. The hypersensitive response elicitor protein or polypeptide of *Erwinia stewartii* is set forth in Ahmad et al., "Harpin is Not

5 Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference.

Hypersensitive response elicitor proteins or polypeptides from

10 *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora* are described in Kaman, et al., "Extracellular Protein Elicitors from Phytophthora: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993), Ricci et al., "Structure and

15 Activity of Proteins from Pathogenic Fungi Phytophthora Eliciting Necrosis and Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989), Ricci et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of *Phytophthora parasitica*," Plant Path., 41:298-307 (1992), Baillreul et al, "A New Elicitor of the Hypersensitive Response in Tobacco: A

20 Fungal Glycoprotein Elicits Cell Death, Expression of Defence Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference.

25 Another hypersensitive response elicitor in accordance with the present invention is from *Clavibacter michiganensis* subsp. *sepedonicus* which is fully described in U.S. Patent Application Serial No. 09/136,625, which is hereby incorporated by reference.

30 The above elicitors are exemplary. Other elicitors can be identified by growing fungi or bacteria that elicit a hypersensitive response under conditions which genes encoding an elicitor are expressed. Cell-free preparations from culture

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supernatants can be tested for elicitor activity (i.e. local necrosis) by using them to infiltrate appropriate plant tissues.

Fragments of the above hypersensitive response elicitor polypeptides or proteins as well as fragments of full length elicitors from other pathogens are 5 encompassed by the present invention.

Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known elicitor protein are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide 10 that can be tested for elicitor activity according to the procedure described below.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino acid sequence of the 15 elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular 20 portions of the protein. These then would be cloned into an appropriate vector for expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and 25 pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

An example of suitable fragments of a hypersensitive response elicitor which do not elicit a hypersensitive response include fragments of the *Erwinia amylovora* hypersensitive response elicitor. Suitable fragments include a C-terminal 30 fragment of the amino acid sequence of SEQ. ID. No. 23, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, or an internal fragment of the amino acid sequence of SEQ. ID. No. 23. The C-terminal fragment of the amino acid

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sequence of SEQ. ID. No. 23 can span the following amino acids of SEQ. ID. No. 23: 169 and 403, 210 and 403, 267 and 403, or 343 and 403. The internal fragment of the amino acid sequence of SEQ. ID. No. 23 can span the following amino acids of SEQ. ID. No. 23: 105 and 179, 137 and 166, 121 and 150, or 137 and 156. Other suitable 5 fragments can be identified in accordance with the present invention.

Another example of a useful fragment of a hypersensitive response elicitor which fragment does not itself elicit a hypersensitive response is the protein fragment containing amino acids 190 to 294 of the amino acid sequence (SEQ. ID. No. 31) for the *Pseudomonas syringae* pv. *syringae* hypersensitive response elicitor. 10 This fragment is useful in imparting disease resistance and enhancing plant growth.

Yet another example of a useful fragment of a hypersensitive response elicitor is the peptide having an amino acid sequence corresponding to SEQ. ID. No. 39. This peptide is derived from the hypersensitive response eliciting glycoprotein of *Phytophthora megasperma* and enhances plant growth.

15 Variants may be made by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure, and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide 20 may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

The fragment of the present invention is preferably in isolated form (i.e. separated from its host organism) and more preferably produced in purified form (preferably at least about 60%, more preferably 80%, pure) by conventional 25 techniques. Typically, the fragment of the present invention is produced but not secreted into the growth medium of recombinant host cells. Alternatively, the protein or polypeptide of the present invention is secreted into growth medium. In the case of unsecreted protein, to isolate the protein fragment, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical treatment, 30 and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to heat treatment and the fragment is separated by centrifugation. The supernatant fraction containing the fragment is subjected to gel filtration in an

appropriately sized dextran or polyacrylamide column to separate the fragment. If necessary, the protein fraction may be further purified by ion exchange or HPLC.

The DNA molecule encoding the fragment of the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccina virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A

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Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria 5 transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression 10 elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

15 Transcription of DNA is dependent upon the presence of a promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promotors differ from those of procaryotic promotors. Furthermore, eucaryotic promotors and accompanying genetic signals may not be recognized in or may not function in a 20 procaryotic system, and, further, procaryotic promotors are not recognized and do not function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called 25 the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct 30 positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

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Promotors vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promotors in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a 5 number of suitable promotors may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promotors such as the T7 phage promotor, *lac* promotor, *trp* promotor, *recA* promotor, ribosomal RNA promotor, the P_R and P_L promotors of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA 10 segments. Additionally, a hybrid *trp-lacUV5* (*tac*) promotor or other *E. coli* promotors produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promotor unless specifically induced. In certain operations, 15 the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene 20 transcription and translation in prokaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promotor, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in 25 *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD- 30 ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the fragment of a hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending 5 upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

The present invention further relates to methods of imparting disease resistance to plants, enhancing plant growth, and/or effecting insect control for plants. These methods involve applying the fragment of a hypersensitive response elicitor 10 polypeptide or protein which does not elicit a hypersensitive response in a non-infectious form to all or part of a plant or a plant seed under conditions effective for the fragment to impart disease resistance, enhance growth, and/or control insects. Alternatively, these fragments of a hypersensitive response elicitor protein or 15 polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to impart disease resistance in plants, to enhance plant growth, and/or to effect insect control.

As an alternative to applying a fragment of a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart disease resistance in plants, to effect plant growth, and/or to control insects on the plants or 20 plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a fragment of a hypersensitive response elicitor polypeptide or protein, which fragment does not elicit a hypersensitive response, and growing the plant under conditions effective to permit that DNA molecule to impart 25 disease resistance to plants, to enhance plant growth, and/or to control insects.

Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a fragment of a hypersensitive response elicitor polypeptide or protein which fragment does not elicit a hypersensitive response can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that 30 DNA molecule to impart disease resistance to plants, to enhance plant growth, and/or to control insects.

The embodiment of the present invention where the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be carried out in a number of ways, including: 1) application of an isolated fragment or 2) application of bacteria which do not cause disease and are transformed with a gene 5 encoding the fragment. In the latter embodiment, the fragment can be applied to plants or plant seeds by applying bacteria containing the DNA molecule encoding the fragment of the hypersensitive response elicitor polypeptide or protein which fragment does not elicit a hypersensitive response. Such bacteria must be capable of secreting or exporting the fragment so that the fragment can contact plant or plant 10 seed cells. In these embodiments, the fragment is produced by the bacteria *in planta* or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.

The methods of the present invention can be utilized to treat a wide variety of plants or their seeds to impart disease resistance, enhance growth, and/or control insects. Suitable plants include dicots and monocots. More particularly, 15 useful crop plants can include: alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, 20 sorghum, and sugarcane. Examples of suitable ornamental plants are: *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

With regard to the use of the fragments of the hypersensitive response elicitor protein or polypeptide of the present invention in imparting disease resistance, 25 absolute immunity against infection may not be conferred, but the severity of the disease is reduced and symptom development is delayed. Lesion number, lesion size, and extent of sporulation of fungal pathogens are all decreased. This method of imparting disease resistance has the potential for treating previously untreatable diseases, treating diseases systemically which might not be treated separately due to 30 cost, and avoiding the use of infectious agents or environmentally harmful materials.

The method of imparting pathogen resistance to plants in accordance with the present invention is useful in imparting resistance to a wide variety of

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pathogens including viruses, bacteria, and fungi. Resistance, *inter alia*, to the following viruses can be achieved by the method of the present invention: *Tobacco mosaic virus* and *Tomato mosaic virus*. Resistance, *inter alia*, to the following bacteria can also be imparted to plants in accordance with present invention:

5 *Pseudomonas solanacearum*, *Pseudomonas syringae* pv. *tabaci*, and *Xanthomonas campestris* pv. *pelargonii*. Plants can be made resistant, *inter alia*, to the following fungi by use of the method of the present invention: *Fusarium oxysporum* and *Phytophthora infestans*.

With regard to the use of the fragments of the hypersensitive response 10 elicitor protein or polypeptide of the present invention to enhance plant growth, various forms of plant growth enhancement or promotion can be achieved. This can occur as early as when plant growth begins from seeds or later in the life of a plant. For example, plant growth according to the present invention encompasses greater 15 yield, increased quantity of seeds produced, increased percentage of seeds germinated, increased plant size, greater biomass, more and bigger fruit, earlier fruit coloration, and earlier fruit and plant maturation. As a result, the present invention provides significant economic benefit to growers. For example, early germination and early maturation permit crops to be grown in areas where short growing seasons would otherwise preclude their growth in that locale. Increased percentage of seed 20 germination results in improved crop stands and more efficient seed use. Greater yield, increased size, and enhanced biomass production allow greater revenue generation from a given plot of land.

Another aspect of the present invention is directed to effecting any 25 form of insect control for plants. For example, insect control according to the present invention encompasses preventing insects from contacting plants to which the hypersensitive response elicitor has been applied, preventing direct insect damage to plants by feeding injury, causing insects to depart from such plants, killing insects proximate to such plants, interfering with insect larval feeding on such plants, preventing insects from colonizing host plants, preventing colonizing insects from 30 releasing phytotoxins, etc. The present invention also prevents subsequent disease damage to plants resulting from insect infection.

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The present invention is effective against a wide variety of insects. European corn borer is a major pest of corn (dent and sweet corn) but also feeds on over 200 plant species including green, wax, and lima beans and edible soybeans, peppers, potato, and tomato plus many weed species. Additional insect larval feeding 5 pests which damage a wide variety of vegetable crops include the following: beet armyworm, cabbage looper, corn ear worm, fall armyworm, diamondback moth, cabbage root maggot, onion maggot, seed corn maggot, pickleworm (melonworm), pepper maggot, tomato pinworm, and maggots. Collectively, this group of insect pests represents the most economically important group of pests for vegetable 10 production worldwide.

The method of the present invention involving application of the fragment of a hypersensitive response elicitor polypeptide or protein, which fragment does not elicit a hypersensitive response, can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots, 15 propagules (e.g., cuttings), etc. This may (but need not) involve infiltration of the fragment of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include high or low pressure spraying, injection, and leaf abrasion proximate to when elicitor application takes place. When treating plant seeds or propagules (e.g., cuttings), in accordance with the application embodiment of 20 the present invention, the fragment of the hypersensitive response elicitor protein or polypeptide, in accordance with present invention, can be applied by low or high pressure spraying, coating, immersion, or injection. Other suitable application procedures can be envisioned by those skilled in the art provided they are able to effect contact of the fragment with cells of the plant or plant seed. Once treated with 25 the fragment of the hypersensitive response elicitor of the present invention, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated in accordance with the present invention, the plants may be treated with one or more applications of the fragment of the hypersensitive response elicitor protein or 30 polypeptide or whole elicitors to impart disease resistance to plants, to enhance plant growth, and/or to control insects on the plants.

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The fragment of the hypersensitive response elicitor polypeptide or protein, in accordance with the present invention, can be applied to plants or plant seeds alone or in a mixture with other materials. Alternatively, the fragment can be applied separately to plants with other materials being applied at different times.

5 A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains a fragment of a hypersensitive response elicitor polypeptide or protein which fragment does not elicit a hypersensitive response in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition contains
10 greater than 500 nM of the fragment.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematicide, and mixtures thereof. Suitable fertilizers include $(\text{NH}_4)_2\text{NO}_3$. An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

15 Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response eliciting fragment can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

20 In the alternative embodiment of the present invention involving the use of transgenic plants and transgenic seeds, a fragment of a hypersensitive response elicitor need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding such a fragment are produced according to procedures well known in the art.

25 The vector described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA. Crossway, Mol. Gen. Genetics, 202:179-85 (1985), which is hereby incorporated by reference. The genetic material may also be transferred into the plant cell using polyethylene glycol. Krens, et al., Nature, 296:72-74 (1982), which is hereby
30 incorporated by reference.

Another approach to transforming plant cells with a gene which imparts resistance to pathogens is particle bombardment (also known as biolistic

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transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby incorporated by reference. Generally, this procedure 5 involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is 10 carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies. 15 Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

The DNA molecule may also be introduced into the plant cells by electroporation. Fromm et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are 20 electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the DNA molecule into plant cells is to 25 infect a plant cell with *Agrobacterium tumefaciens* or *A. rhizogenes* previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration 30 medium without antibiotics at 25-28°C.

Agrobacterium is a representative genus of the Gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy

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root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, 5 assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by Agrobacterium and is stably integrated into the plant genome. J. Schell, Science, 10 237:1176-83 (1987), which is hereby incorporated by reference.

After transformation, the transformed plant cells must be regenerated. Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by 15 reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

20 Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form 25 plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and 30 repeatable.

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After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves 5 can be cultivated in accordance with conventional procedure with the presence of the gene encoding the fragment of the hypersensitive response elicitor resulting in disease resistance, enhanced plant growth, and/or control of insects on the plant. Alternatively, transgenic seeds or propagules (e.g., cuttings) are recovered from the 10 transgenic plants. The seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under conditions effective to impart 15 disease resistance to plants, to enhance plant growth, and/or to control insects. While not wishing to be bound by theory, such disease resistance, growth enhancement, and/or insect control may be RNA mediated or may result from expression of the polypeptide or protein fragment.

When transgenic plants and plant seeds are used in accordance with the present invention, they additionally can be treated with the same materials as are used to treat the plants and seeds to which a fragment of a hypersensitive response elicitor in accordance with the present invention is applied. These other materials, including 20 a fragment of a hypersensitive response elicitor in accordance with the present invention, can be applied to the transgenic plants and plant seeds by the above-noted procedures, including high or low pressure spraying, injection, coating, and immersion. Similarly, after plants have been propagated from the transgenic plant seeds, the plants may be treated with one or more applications of the fragment of a 25 hypersensitive response elicitor in accordance with the present invention to impart disease resistance, enhance growth, and/or control insects. Such plants may also be treated with conventional plant treatment agents (e.g., insecticides, fertilizers, etc.).

EXAMPLES

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Example 1 - Bacterial Strains and Plasmids

Escherichia coli strains used in the following examples include DH5 α and BL21(DE3) purchased from Gibco BRL (Grand Island, N.Y.) and Stratagene

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(La Jolla, CA), respectively. The pET28(b) vector was purchased from Novagen (Madison, WI). Eco DH5 α /2139 contained the complete *hrpN* gene. The 2139 construct was produced by D. Bauer at Cornell University. The *hrpN* gene was cleaved from the 2139 plasmid by restriction enzyme digestion with HindIII, then purified from an agarose gel to serve as the DNA template for PCR synthesis of truncated *hrpN* clones. These clones were subsequently inserted into the (His)₆ vector pET28(b) which contained a Kan^r gene for selection of transformants.

Example 2 - DNA Manipulation

Example 3 - Fragmentation of *hrpN* Gene

25 A series of N-terminal and C-terminal truncated *hrpN* genes and
internal fragments were generated via PCR (Fig. 1). The full length *hrpN* gene was
used as the DNA template and 3' and 5' primers were designed for each truncated
clone (Fig. 2). The 3' primers contained an NdeI enzyme cutting site which contained
the start codon ATG (methionine) and the 5' primers contained the stop codon TAA
30 and a HindIII enzyme cutting site for ligation into the pET28(b) vector. PCR was
carried out in 0.5 ml tubes in a GeneAmpTM 9700 (Perkin-Elmer, Foster City, CA).
45 μ l of SupermixTM (Life Technology, Gaithersburg, MD) were mixed with 20
pmoles of each pair of DNA primers, 10 ng of full length harpin DNA, and deionized

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H₂O to a final volume of 50 µl. After heating the mixture at 95°C for 2 min, the PCR was performed for 30 cycles at 94°C for 1 min, 58°C for 1 min and 72°C for 1.5 min. The PCR products were verified on a 6% TBE gel (Novex, San Diego, CA).

Amplified DNA was purified with the QIAquick PCR purification kit, digested with

5 Nde I and Hind III at 37°C for 5 hours, extracted once with

phenol:chloroform:isoamylalcohol (25:25:1) and precipitated with ethanol. 5 µg of pET28(b) vector DNA were digested with 15 units of Nde I and 20 units of Hind III at 37°C for 3 hours followed with CIAP treatment to reduce the background resulting from incomplete single enzyme digestion. Digested vector DNA was purified with

10 the QIAquick PCR purification kit and directly used for ligation. Ligation was carried out at 14-16°C for 5-12 hours in a 15 µl mixture containing ca. 200 ng of digested pET28(b), 30 ng of targeted PCR fragment, and 1 unit T4 DNA ligase. 5 - 7.5 µl of ligation solution were added to 100 µl of DH5 α competent cells in a 15 ml Falcon tube and incubated on ice for 30 min. After a heat shock at 42°C for 45 seconds, 0.9

15 ml SOC solution or 0.45 ml LB media were added to each tube and incubated at 37°C for 1 hour. 20, 100, and 200 µl of transformed cells were placed onto LB agar with 30 µg/ml of kanamycin and incubated at 37°C overnight. Single colonies were transferred to 3 ml LB-media and incubated overnight at 37°C. Plasmid DNA was prepared from 2 ml of culture with the QIAprep Miniprep kit (QIAGEN, Hilden, Germany).

20 The DNA from the transformed cells was analyzed by restriction enzyme digestion or partial sequencing to verify the success of the transformations. Plasmids with the desired DNA sequence were transferred into the BL21 strain using the standard chemical transformation method as indicated above. A clone containing the full length harpin protein in the pET28(b) vector was generated as a positive control, 25 and a clone with only the pET28(b) vector was generated as a negative control.

Example 4 - Expression of Hypersensitive Response Elicitor Truncated Proteins

30 *Escherichia coli* BL21(DE3) strains containing the hrpN clones were grown in Luria broth medium (5g/L Difco Yeast extract, 10 g/L Difco Tryptone, 5 g/L NaCl, and 1 mM NaOH) containing 30 µg/ml of kanamycin at 37°C overnight. The bacteria were then inoculated into 100 volumes of the same medium and grown at

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37°C to an OD₆₂₀ of 0.6-0.8. The bacteria were then inoculated into 250 volumes of the same medium and grown at 37°C to an OD₆₂₀ of ca. 0.3 or 0.6-0.8. One milli molar IPTG was then added and the cultures grown at 19°C overnight (ca. 18 hours). Not all of the clones were successfully expressed using this strategy. Several of the 5 clones had to be grown in Terrific broth (12 g/L Bacto Tryptone, 24 g/L Bacto yeast, 0.4% glycerol, 0.17 M KH₂PO₄, and 0.72 K₂HPO₄), and/or grown at 37°C after IPTG induction, and/or harvested earlier than overnight (Table 1).

Table 1: Expression of hypersensitive response elicitor truncated proteins

10

Fragment	amino acids (SEQ. ID. No. 23)	Growth medium	Induction O.D.	Expression temp.	Harvest time
1 (+ control)	1-403	LB	ca. 0.3 or 0.6-0.8	19°C or 25°C	16-18 hr
2 (+ control)	-	LB and TB	ca. 0.3 or 0.6-0.8	19°C and 37°C	16-18 hr
3	105-403	LB	0.6-0.8	19°C	16-18 hr
4	169-403	TB	ca. 0.3	19°C	16-18 hr
5	210-403	LB or M9ZB	0.6-0.8	19°C	16-18 hr
6	257-403	LB or M9ZB	0.6-0.8	19°C	16-18 hr
7	343-403	LB	ca. 0.3	19°C	5 hr
8	1-75	TB	ca. 0.3	37°C	16-18 hr
9	1-104	TB	ca. 0.3	37°C	16-18 hr
10	1-168	TB	ca. 0.3	37°C	16-18 hr
11	1-266	LB	ca. 0.3	37°C	4 hr
12	1-342	LB	0.6-0.8	19°C	16-18 hr
13	76-209	LB	ca. 0.3	37°C	5 hr
14	76-168	TB or LB	ca. 0.3	37°C	3 hr or 16-18 hr
15	105-209	M9ZB	ca. 0.3	37°C	3 hr
16	169-209		no expression		
17	105-168	LB	ca. 0.3	37°C	3-5 hr
18	99-209	LB	ca. 0.3	37°C	3 hr
19	137-204	LB	ca. 0.3	37°C	3 hr
20	137-180	LB	ca. 0.3	37°C	16-18 hr.
21	105-180	LB	ca. 0.3	37°C	3 hr
22	150-209		no expression		
23	150-180		no expression		

Example 5 - Small Scale Purification of Hypersensitive Response Elicitor Truncated Proteins (Verification of Expression)

15

A 50 ml culture of a hrpN clone was grown as above to induce expression of the truncated protein. Upon harvesting of the culture, 1.5 ml of the cell

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suspension were centrifuged at 14,000 rpm for 5 minutes, re-suspended in urea lysis buffer (8 M urea, 0.1 M Na₂HPO₄, and 0.01 M Tris -- pH 8.0), incubated at room temperature for 10 minutes, then centrifuged again at 14,000 rpm for 10 minutes, and the supernatant saved. A 50 μ l aliquot of a 50% slurry of an equilibrated (His)₆-

5 binding nickel agarose resin was added to the supernatant and mixed at 4°C for one hour. The nickel agarose was then washed three times with urea washing buffer (8 M urea, 0.1 M Na₂HPO₄, and 0.01 M Tris -- pH 6.3), centrifuging at 5,000 rpm for five minutes between washings. The protein was eluted from the resin with 50 μ l of urea elution buffer (8 M urea, 0.1 M Na₂HPO₄, 0.01 M Tris, and 0.1 M EDTA -- pH 6.3).

10 The eluate was run on a 4-20%, a 16%, or a 10-20% Tris-Glycine pre-cast gel depending upon the size of the truncated protein to verify the expression.

Example 6 - Induction of HR in Tobacco

A 1.5 ml aliquot from the 50 ml cultures grown for small scale purification of the truncated proteins was centrifuged at 14,000 rpm for four minutes and re-suspended in an equal volume of 5 mM potassium phosphate buffer, pH 6.8. The cell suspension was sonicated for ca. 30 seconds then diluted 1:2 and 1:10 with phosphate buffer. Both dilutions plus the neat cell lysate were infiltrated into the fourth to ninth leaves of 10-15 leaf tobacco plants by making a hole in single leaf panes and infiltrating the bacterial lysate into the intercellular leaf space using a syringe without a needle. The HR response was recorded 24-48 hr post infiltration. Tobacco (*Nicotiana tabacum* v. Xanthi) seedlings were grown in an environmental chamber at 20-25°C with a photoperiod of 12-h light /12-h dark and ca. 40% RH. Cell lysate was used for the initial HR assays (in order to screen the truncated proteins for HR activity) as the small scale urea purification yielded very little protein which was denatured due to the purification process.

Example 7 - Large Scale Native Purification of Hypersensitive Response Elicitor Truncated Proteins for Comprehensive Biological Activity Assays

30 Six 500 ml cultures of a hrpN clone were grown as described earlier to induce expression of the truncated protein. Upon harvesting of the culture, the cells were centrifuged at 7,000 rpm for 5 minutes, re-suspended in imidazole lysis buffer (5

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mM imidazole, 0.5 M NaCl, 20 mM Tris) plus Triton X-100 at 0.05% and lysozyme at 0.1 mg/ml, incubated at 30°C for 15 minutes, sonicated for two minutes, centrifuged again at 15,000 rpm for 20 minutes, and the supernatant was saved. A 4 ml aliquot of a 50% slurry of an equilibrated (His)₆-binding nickel agarose resin was 5 added to the supernatant and mixed at 4°C for ca. four hours. The nickel agarose was then washed three times with imidazole washing buffer (20 mM imidazole, 0.5 M NaCl, and 20 mM Tris), centrifuging at 5,000 rpm for five minutes between washings, then placed in a disposable chromatography column. The column was centrifuged at 10 1100 rpm for one minute to remove any residual wash buffer and then the protein was eluted from the resin with 4 ml of imidazole elution buffer (1 M imidazole, 0.5 M NaCl, and 20 mM Tris) by incubating the column with the elution buffer for ten minutes at room temperature and then centrifuging the column at 1100 rpm for one minute. The eluate was run on a 4-20%, a 16%, or a 10-20% Tris-Glycine pre-cast 15 gel depending upon the size of the truncated protein to verify the expression. The concentration of the proteins was determined by comparison of the protein bands with a standard protein in the Mark 12 molecular weight marker.

Example 8 - Large Scale Urea Purification of Hypersensitive Response Elicitor Truncated Proteins For Comprehensive Biological Activity Assay

20 The procedure was the same as the large scale native purification except that urea lysis buffer, washing buffer, and elution buffer were used, and the cells were not sonicated as in the native purification. After purification, the protein was renatured by dialyzing against lower and lower concentrations of urea over an 25 eight hour period, then dialyzing overnight against 10 mM Tris/20 mM NaCl. The renaturing process caused the N-terminal proteins to precipitate. The precipitated 1-168 protein was solubilized by the addition of 100 mM Tris-HCl at pH 10.4 then heating the protein at 30°C for ca. one hour. The concentration of the protein was determined by comparison of the protein bands with a standard protein in the Mark 12 30 molecular weight marker. The 1-75 and 1-104 protein fragments were not successfully solubilized using this strategy so they were sonicated in 100 mM Tris-HCl at pH 10.4 to solubilize as much of the protein as possible and expose the active sites of the protein for the biological activity assays.

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Example 9 – Induction of Growth Enhancement (GE)

Sixty tomato (*Lycopersicon spp.* cv. Marglobe) seeds were soaked
5 overnight in 10 and 20 µg/ml of the truncated protein diluted with 5mM potassium
phosphate buffer, pH 6.8. The next morning, the sixty seeds were sown in three pots
and 12-15 days later and again 18-20 days later the heights of the 10 tallest tomato
plants per pot were measured and compared with the heights of the control plants
treated only with phosphate buffer. Analyses were done on the heights to determine if
10 there was a significant difference in the height of the plants treated with the truncated
proteins compared with the buffer control, and thereby determine whether the proteins
induced growth enhancement.

Example 10 – Induction of Systemic Acquired Resistance (SAR)

15 Three tobacco (*Nicotiana tabacum* cv. Xanthi) plants with 8-12 leaves
(ca. 75 day old plants) were used in the assay. One leaf of the tobacco plants was
covered up and the rest of the leaves were sprayed with ca. 50 ml of a 20 µg/ml
solution of the truncated proteins diluted with 5mM potassium phosphate buffer. Five
20 to seven days later two leaves (the unsprayed leaf and the sprayed leaf opposite and
just above the unsprayed leaf) were inoculated with 20 µl of a 1.8 µg/ml solution of
TMV along with a pinch of diatomaceous earth by rubbing the mixture along the top
surface of the leaves. The TMV entered the plants through tiny lesions made by the
diatomaceous earth. Ca. 3-4 days post TMV inoculation, the number of TMV lesions
25 was counted on both leaves compared with the number of lesions on the negative
control buffer treated leaves. Analyses were done to determine the efficacy of
reducing the number of TMV lesions by the protein fragments compared to the buffer
control. Percentage of efficacy was calculated as: Reduction in TMV lesions (%
efficacy) = $100 \times (1 - \text{mean } \# \text{ of lesions on treated leaves} / \text{mean } \# \text{ of lesions on buffer control leaves})$.
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Example 11 - Expression of Hypersensitive Response Elicitor Truncated Proteins

The small scale expression and purification of the fragment proteins was done to screen for expression and HR activity (Table 2).

5

Table 2

Expression and HR activity of hypersensitive response elicitor truncated proteins (small scale screening)

Fragment #	Amino Acids (SEQ. ID. No. 23)	Expression	HR activity
1(+control)	1-403	+	+
2(- control)	-	background protein only	-
3	105-403	+	+
4	169-403	+	-
5	210-403	+	-
6	267-403	+	-
7	343-403	+/-	-
8	1-75	+	-
9	1-104	+	+/-
10	1-168	+	+
11	1-266	+	+
12	1-342	+	+
13	76-209	+	+
14	76-168	+	-
15	105-209	+	+
16	169-209	-	-
17	105-168	+	-
18	99-209	+	+
19	137-204	+	+
20	137-180	+	+
21	105-180	+	+
22	150-209	-	-
23	150-180	-	-

10

All of the cloned fragment proteins were expressed at varying levels except for three small fragments (amino acids 169-209, 150-209, and 150-180). Fragments 210-403 and 267-403 were expressed very well, yielding a high concentration of protein from a small scale purification, resulting in a substantial protein band on SDS gel electrophoresis. Other fragments (such as a.a. 1-168 and 1-104) produced much less protein, resulting in faint protein bands upon electrophoresis. It was difficult to determine whether fragment 343-403, the smallest C-terminal protein, was expressed, as there were several background proteins apparent on the gel, in addition to the suspected 343-403 protein. The positive and negative control proteins, consisting of

15

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the full length hypersensitive response elicitor protein and only background proteins, respectively, were tested for expression and HR activity as well.

15 The large scale expression and purification of the fragment proteins was done to determine the level of expression and titer of the HR activity (Table 3).

20 5

Table 3

Expression level and HR titer of hypersensitive response elicitor truncated proteins (large scale purification)

Fragment #	Amino acids (SEQ. ID. No. 23)	Expression	HR titer
1 (+ control)	1-403	3.7 mg/ml	5-7 µg/ml
2 (- control)	-	-	1:2 dilution
4	169-403	2 mg/ml	-
5	210-403	5 mg/ml	-
6	267-403	4 mg/ml	-
7	343-402	200 µg/ml	-
8	1-75	50 µg/ml	-
9	1-104	50 µg/ml	3 µg/ml (1:16 dilution)
10	1-168	1 mg/ml	1 µg/ml
13	76-209	2.5 mg/ml	5 µg/ml
14	76-168	2 mg/ml	-
15	105-209	5 mg/ml	5-10 µg/ml
17	105-168	250 µg/ml	-
19	137-204	3.6 mg/ml	3.5 µg/ml
20	137-180	250 µg/ml	16 µg/ml

15 The truncated proteins deemed to be the most important in characterizing the hypersensitive response elicitor were chosen for large scale expression. The positive control (full length hypersensitive response elicitor) was expressed at a relatively high level at 3.7 mg/ml. All of the C-terminal proteins were expressed at relatively high levels from 2-5 mg/ml, except for fragment 343-403 as discussed earlier. The N-terminal fragments were expressed very well also; however, during the purification process, the protein precipitated and very little was resolubilized. The concentrations 20 in Table 3 reflect only the solubilized protein. The internal fragments were expressed in the range of 2-3.6 mg/ml. It was extremely difficult to determine the concentration of fragment 105-168 (it was suspected that the concentration was much higher than indicated), as the protein bands on the SDS gel were large, but poorly stained. The

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negative control contained several background proteins as expected, but no obviously induced dominant protein.

Example 12 - Induction of HR in Tobacco

5 The full length positive control protein elicited HR down to only 5-
7 μ g/ml. The negative control (pET 28) imidazole purified "protein" - which
contained only background proteins - elicited an HR response down to the 1:2
dilution, which lowered the sensitivity of the assay as the 1:1 and 1:2 dilutions could
10 not be used. This false HR was likely due to an affinity of the imidazole used in the
purification process to bind to one or several of the background proteins, thereby not
completely dialyzing out. Imidazole at a concentration of ca. 60 mM did elicit a false
HR response.

15 One definitive domain encompassing a small internal region of the
protein from a.a. 137-180 (SEQ. ID. No. 23), a mere 44 a.a, is identified as the
smallest HR domain. The other potential HR domain is thought to be located in the
N-terminus of the protein from a.a. 1-104 (possibly a.a. 1-75) (SEQ. ID. No. 23). It
was difficult to confirm or narrow down the N-terminus HR domain due to the
difficulties encountered in purifying these fragment proteins. The N-terminus
20 fragment proteins had to be purified with urea as no protein was recovered when the
native purification process was used. Consequently, these proteins precipitated during
the renaturing process and were difficult or nearly impossible to get back into
solution, thereby making it hard to run the proteins through the HR assay, as only
soluble protein is able to elicit HR. Difficulty narrowing the N-terminus HR domain
25 was only compounded by the fact that the negative control elicited false HR at the low
dilution levels thereby reducing the sensitivity of the assay.

30 Surprisingly, when the internal HR domain was cleaved between a.a.
168 and 169 (fragments 76-168 and 105-168) (SEQ. ID. No. 23) the fragment lost its
HR activity. This suggests that the HR activity of fragment 1-168 (SEQ. ID. No. 23)
should not be attributed to the internal HR domain, but rather to some other domain,
leading to the assumption that there was likely a second HR domain to be found in the
N-terminal region of the protein. However, as discussed earlier it was difficult to
confirm this assumption.

5 The hypersensitive response elicitor C-terminus (a.a. 210-403 (SEQ. ID. No. 23)) did not contain an HR domain. It did not elicit HR at a detectable level using the current HR assay. Even the large C-terminal fragment from a.a. 169-403 (SEQ. ID. No. 23) did not elicit HR even though it contained part of the internal HR domain. As stated above, cleaving the protein between amino acids 168 and 169 (SEQ. ID. No. 23) causes a loss of HR activity.

10 Because some of the small cloned proteins with 61 a.a. or less were not expressed, several oligopeptides were synthesized with 30 a.a. to narrow down the functional region of the internal HR domain. The oligopeptides were synthesized within the range of a.a. 121-179 (SEQ. ID. No. 23). However, these oligos did not elicit HR. It was not expected that there would be an HR from oligos 137-166, 121-150, and 137-156 (SEQ. ID. No. 23) as these fragments did not contain the imperative amino acids 168 and 169 (SEQ. ID. No. 23). It was expected that the oligo 150-179 (SEQ. ID. No. 23) would elicit an HR. It is possible that 30 a.a. is too small for the protein to elicit any activity due to a lack of folding and, therefore, a lack of binding or that during the synthesis of the peptides important amino acids were missed (either in the process, or simply by the choice of which 30 amino acids to synthesize) and, therefore, the fragments would not be able to elicit HR.

20 **Example 13 – Induction of Plant Growth Enhancement (PGE)**

25 The C-terminal fragments enhanced the growth of tomato by 9% to 21%. The N-terminal fragments enhanced the growth of tomato by 4% to 13%. The internal fragments enhanced growth by 9% to 20%. The 76-209 fragment enhanced growth by 18% at a concentration of 60 μ g/ml, but not at the typical 20 μ g/ml. This was attributed to the inaccuracy of the quantification process (Table 4).

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Table 4

Fragment #	Amino acids	PGE ht>buffer @ 10 µg/ml	PGE ht>buffer @ 20 µg/ml
1 (+ control)	1-403	12%	11%
2 (- control)	-	-3%	-2%
4	169-403	9%	12%
5	210-403	13%	14% 16% @ 40µg/ml
6	267-403	21%	21% 23% @ 40µg/ml
7	343-403	7%	7%
9	1-104	4%	8%
10	1-168	13%	5%
13	76-209	7%	4% 18% @ 60µg/ml
14	76-168	-18%	-20%
15	105-209	14%	19%
17	105-168	19%	16%
19	137-204	11%	13%
20	137-180	--	9%

*A height greater than 10% above the buffer control was necessary to pass the PGE assay.

5

The oligopeptides enhanced growth from 7.4% to 17.3% (Table 5).

10

Table 5

Fragment	Amino acids	Expression	HR titer	TMV efficacy	PGE ht>buffer
oligo	150-179	NA	-	72.9%	10.1%
oligo	137-166	NA	-	61.2%	12.0%
oligo	121-150	NA	-	60.0%	17.3%
oligo	137-156	NA	-	-87.7%	7.4%

The data suggests that there is more than one PGE domain, although the C-terminal and internal domains appear to be dominant over the N-terminal domain, as the N-terminal fragments enhanced growth the least amount.

15

Example 14 – Induction of Systemic Acquired Resistance (SAR)

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All of the hypersensitive response elicitor fragments tested to date appear to have 60% efficacy or greater, except for the oligopeptide 137-156 (Tables 5 and 6).

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Table 6

Fragment #	Amino acids	Efficacy of TMV control
1 (+ control)	1-403	84% & 72%
2 (- control)	-	40% & 31%
4	169-403	64% & 79%
5	210-403	77% and 78%
6	267-403	70% and 72%
9	1-104	82%
10	1-168	69%
13	76-209	44% and 84%
14	76-168	83% & 87%
15	105-209	57% and 67%
17	105-168	89%
19	137-204	89% & 77%
20	137-180	64% & 58%

5

These data suggest that there are multiple SAR domains within the protein.

Example 15 – Relationship Between HR, PGE, and SAR

10

It is clear that the hypersensitive response activity is separable from the plant growth enhancement activity. The C-terminal fragments clearly enhance the growth of tomato by ca. 20% at a concentration of only 20 µg/ml, but these same fragments were not able to elicit HR in tobacco, even at higher concentrations than 200 µg/ml. The SAR activity also appears to be separable from the HR activity. This finding is highly significant for future work on transgenic applications of the hypersensitive response elicitor technology. The fragments that induce PGE and/or SAR but do not elicit HR will be imperative for this technology, as constitutive expression of even low levels of an HR elicitor might kill a plant.

15

Example 16 - Non-HR Eliciting Fragments Derived from the Hypersensitive Response Elicitor from *Pseudomonas syringae* pv. *syringae* Induce Resistance in Tobacco to TMV and Promote the Growth of Tomato

20

To test whether non-HR eliciting fragments derived from HrpZ, the hypersensitive response elicitor from *Pseudomonas syringae* pv. *syringae*, is able to induce disease resistance, several fragment constructs were made and the expressed

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fragment proteins were tested for HR elicitation and disease resistance induction in tobacco and growth promotion in tomato.

The following segments of *hrpZ*, the gene encoding the hypersensitive response elicitor from *Pseudomonas syringae* pv. *syringae*, were amplified by PCR

5 using Pfu Turbo (Stratagene): Regions coding for amino acids 152-190, aa 152-294, aa 190-294, aa 301-341, and full length HrpZ (aa 1-341). The DNA fragments were cloned into pCAL-n (Stratagene) to create C-terminal fusion proteins to the

calmodulin-binding peptide. pCAL-n was chosen, because the fusion protein could be easily and gently purified on calmodulin resin. The DNA was transformed into *E.*

10 *coli* DH5 α , and the correct clones were identified. The clones were then transferred to *E. coli* BLR DE3 for protein expression. The bacteria were grown in Terrific Broth to an OD₆₂₀ of 0.8-1.0. Protein expression was then induced with IPTG and the bacteria were incubated for an additional 3 h. All of the HrpZ fragments were able to be expressed this way.

15 Amino acid fragments 152-294 and 190-294 were chosen for further analysis and characterization. It was expected that the fragment 152-294 contained a domain that elicited the HR, while fragment 190-294 contained no domain that elicited the HR. The cultures were spun down, and the bacteria resuspended in 40 ml of 10 mM Tris pH 8.0. Twenty μ l of antifoam and 40 μ l of 200 mM PMSF were 20 added, and the bacteria was sonicated to break open the cells. The bacterial debris was removed by centrifugation, and the supernatant was placed in a boiling water bath for 10 min. The precipitate was removed by centrifugation and the supernatant, a crude protein preparation, was retained for tests.

25 Fifteen μ l of each supernatant was run on a gel and stained to determine if the protein was present. It was estimated that about five times as much of the 152-294 fragment was present as the 190-294 fragment. Several dilutions of each preparation were infiltrated into tobacco leaves on two plants for HR tests (Table 7). As shown in Table 7, the 152-294 fragment elicited an HR, but the 190-294 fragment did not.

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Table 7
HR test results of HrpZ fragments

<u>HrpZ Fragment</u>	<u>Dilution of Fragment Preparation^a</u>			
	<u>1:2</u>	<u>1:5</u>	<u>1:25</u>	<u>1:125</u>
152-294	+,+	+,+	+,+	-,
<u>190-294</u>	—	—	—	—

^a The preparations were diluted with MilliQ water.

^b The results are indicated for each of two plants. +, HR; -, no HR.

10

The fragment preparations were then tested for inducing resistance to TMV and for growth enhancement. Due to the difference in concentration of the HrpZ fragments, the 152-294 preparation was diluted 40-fold and the 190-294 preparation was diluted 8-fold. The results showed that the 190-294 aa fragment reduced the number of TMV lesions by 85% in comparison to buffer controls (Table 8). In contrast, the 152-294 aa fragment reduced the number of TMV lesions by only 55%. As also shown in Table 8, plants treated with the 152-294 aa fragment grew 4.64% more than buffer treated plants, while plants treated with the 190-294 aa fragment grew 2.62% more than the buffer treated plants.

15

20

Table 8
HR test, TMV, and PGE test results

	<u>HrpZ Fragment</u>	<u>HR elicitation^a</u>	<u>TMV (% efficacy)^b</u>	<u>PGE(% > buffer ht)^c</u>
25	152-294	+	54.64	4.64
	<u>190-294</u>	—	85.25	2.62

^a +, elicits HR in tobacco leaves; —, no HR in tobacco leaves.

^b % reduction in TMV lesions in unsprayed leaf of tobacco.

30

^c % greater height than buffer sprayed plants.

35

The results of these tests show that amino acids 152-190 appear to be involved in HR elicitation, because their removal eliminated the ability to elicit the HR. Both fragment preparations achieved disease control and growth enhancement. Thus, the ability to elicit the HR is not the determining factor for reduction in TMV infection and growth enhancement.

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Example 17 - Use of 13 Amino Acid Peptide Derived from *Phytophthora megasperma* Stimulates Tomato Seedling Growth

Parsley leaves develop a typical resistance reaction against the soybean 5 pathogen *Phytophthora megasperma* comprising hypersensitive cell death, defense related gene activation, and phytoalexin formulation. Several years ago, a 42 kDa glycoprotein elicitor was purified from the fungal culture filtrate of *Phytophthora megasperma* (Parker et al., "An Extracellular Glycoprotein from *Phytophthora megasperma* f.sp. *glycinea* Elicits Phytoalexin Synthesis in Cultured Parsley Cells and 10 Protoplasts," *Mol. Plant Microbe Interact.* 4:19-27 (1991), which is hereby incorporated by reference). Then, an oligopeptide of 13 amino acid was identified within the 42 kDa glycoprotein. The 13 amino acids peptide appeared to have similar 15 biological activity as that of the full length glycoprotein (42 kDa). It is sufficient to elicit a complex defense response in parsley cells including H+/Ca2+ influxes, K+/Cl- effluxes, active oxygen production, SAR gene induction, and phytoalexin compound 20 accumulation (Nurnberger et al., "High Affinity Binding of a Fungal Oligopeptide Elicitor to Parsley Plasma Membranes Triggers Multiple Defense Response," *Cell* 78:449-460 (1994), which is hereby incorporated by reference).

To test if the 13 amino acid peptide derived from the 42 kDa protein 25 also enhanced plant growth, 20 mg of the oligopeptide was synthesized from Biosynthesis Corp. The synthesized sequence of the peptide is NH2-Val-Trp-Asn-Gln-Pro-Val-Arg-Gly-Phe-Lys-Val-Tyr-Glu-COOH (SEQ. ID. No. 39). The synthesized peptide was resuspended in 10 ml of 5 mM potassium phosphate buffer and, then, diluted to 1 and 100 ng/ml with the same buffer. About 100 tomato seeds (variety, Marglobe) were submerged in 20 ml of peptide solution overnight. The soaked seeds were planted in an 8 inch pot with artificial soil. Seeds soaked in the buffer without the peptide were used as a control. After seedlings emerged and the first two true leaves fully expanded, the height of the tomato seedlings was recorded. The peptide was not able to elicit the HR in tobacco and other tested plants. 30 However, it had a profound effect on plant growth promotion. Table 9 shows that tomato seedlings treated with the peptide increased 12.6 % in height, indicating that the fungal peptide derived from the 42 kDa glycoprotein can promote tomato seedling growth. Extended studies showed that the peptide also had similar growth

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effect in other crops including tobacco. Similar growth promotion effects were achieved by plants sprayed with the peptide solution.

Table 9

5	Treatment	Height of seedlings (cm)					Average (cm)	% Change
10	Buffer	6.0 5.5	6.0 5.5	6.0 5.0	5.5 5.0	5.5 5.5	5.55	
15	Peptide Solution (100ng/ml)	6.5 6.0	6.0 6.0	6.5 6.0	6.5 6.0	6.5 6.5	6.25	12.6

15 Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

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WHAT IS CLAIMED:

1. An isolated fragment of a hypersensitive response elicitor protein or polypeptide, wherein said fragment does not elicit a hypersensitive response but has other activity in plants.

2. An isolated fragment according to claim 1, wherein the hypersensitive response elicitor protein or polypeptide is derived from an *Erwinia*, *Pseudomonas*, *Xanthomonas*, or *Phytophthora*.

10

3. An isolated fragment according to claim 2, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*.

15

4. An isolated fragment according to claim 3, wherein the fragment is selected from the group consisting of a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, and an internal fragment of the amino acid sequence of SEQ. ID. No. 23.

20

5. An isolated fragment according to claim 4, wherein the fragment is a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 169 and 403, 210 and 403, 267 and 403, or 343 and 403.

25

6. An isolated fragment according to claim 4, wherein the fragment is an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23.

30

7. An isolated fragment according to claim 4, wherein the fragment is an internal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 105 and 179, 137 and 166, 121 and 150, or 137 and 156.

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8. An isolated fragment according to claim 2, wherein the hypersensitive response elicitor is derived from *Pseudomonas syringae*.

5 9. An isolated fragment according to claim 8, wherein the fragment contains amino acids 190 to 294 of SEQ. ID. No. 31.

10. 10. An isolated DNA molecule encoding a fragment according to claim 1.

10 11. An isolated DNA molecule according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from an *Erwinia* *Pseudomonas*, *Xanthomonas*, or *Phytophthora*.

15 12. An isolated DNA molecule according to claim 11, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*.

20 13. An isolated DNA molecule according to claim 12, wherein the fragment is selected from the group consisting of a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, and an internal fragment of the amino acid sequence of SEQ. ID. No. 23.

25 14. An isolated DNA molecule according to claim 12, wherein the fragment is a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 169 and 403, 210 and 403, 267 and 403, or 343 and 403.

30 15. An isolated DNA molecule according to claim 12, wherein the fragment is an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23.

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16. An isolated DNA molecule according to claim 12, wherein the fragment is an internal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 105 and 179, 137 and 166, 121 and 150, or 137 and 156.

5

17. An isolated DNA molecule according to claim 11, wherein the hypersensitive response elicitor is derived from *Pseudomonas syringae*.

18. An isolated DNA molecule according to claim 18, wherein the fragment contains amino acids 190 to 294 of SEQ. ID. No. 31.

19. An expression system transformed with a DNA molecule according to claim 10.

15 20. An expression system according to claim 19, wherein said DNA molecule is in proper sense orientation and correct reading frame.

21. A host cell transformed with a DNA molecule according to claim 10.

20 22. A host cell according to claim 21, wherein the host cell is selected from the group consisting of a plant cell and a bacterial cell.

25 23. A host cell according to claim 21, wherein the DNA molecule is transformed with an expression system.

24. A transgenic plant transformed with the DNA molecule of claim 10.

30 25. A transgenic plant according to claim 24, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive,

- 64 -

cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

5

26. A transgenic plant according to claim 24, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

10

27. A transgenic plant seed transformed with the DNA molecule of claim 10.

15

28. A transgenic plant seed according to claim 27, wherein the plant seed is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

20

29. A transgenic plant seed according to claim 27, wherein the plant seed is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

25

30. A method of imparting disease resistance to plants comprising: applying a fragment of a hypersensitive response elicitor protein or polypeptide, which fragment does not elicit a hypersensitive response, in a non-infectious form to a plant or plant seed under conditions effective to impart disease resistance.

30

31. A method according to claim 30, wherein plants are treated during said applying.

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32. A method according to claim 30 wherein plant seeds are treated during said applying, said method further comprising:

5 planting the seeds treated with the fragment of the hypersensitive response elicitor in natural or artificial soil and propagating plants from the seeds planted in the soil.

33. A method of enhancing plant growth comprising:

10 applying a fragment of a hypersensitive response elicitor protein or polypeptide, which fragment does not elicit a hypersensitive response, in a non-infectious form to a plant or plant seed under conditions effective to enhance plant growth.

34. A method according to claim 33, wherein plants are treated 15 during said applying.

35. A method according to claim 33, wherein plant seeds are treated during said applying, said method further comprising:

20 planting the seeds treated with the fragment of the hypersensitive response elicitor in natural or artificial soil and propagating plants from the seeds planted in the soil.

36. A method of insect control for plants comprising:

25 applying a fragment of a hypersensitive response elicitor protein or polypeptide, which fragment does not elicit a hypersensitive response, in a non-infectious form to a plant or plant seed under conditions effective to control insects.

37. A method according to claim 36, wherein plants are treated 30 during said applying.

38. A method according to claim 36, wherein plant seeds are treated during said applying, said method further comprising:

- 66 -

planting the seeds treated with the fragment of the hypersensitive response elicitor in natural or artificial soil and propagating plants from the seeds planted in the soil.

5 39. A method of imparting disease resistance to plants comprising:
 providing a transgenic plant or plant seed transformed with a
DNA molecule which encodes a fragment of a hypersensitive response elicitor protein
or polypeptide, which fragment does not elicit a hypersensitive response, and
 growing the transgenic plant or transgenic plants produced
10 from the transgenic plant seeds under conditions effective to impart disease resistance.

15 40. A method according to claim 39, wherein a transgenic plant is
 provided.

20 41. A method according to claim 39, wherein a transgenic plant
 seed is provided.

25 42. A method of enhancing plant growth comprising:
 providing a transgenic plant or a plant seed transformed with a
DNA molecule which encodes a fragment of a hypersensitive response elicitor protein
or polypeptide, which fragment does not elicit a hypersensitive response, and
 growing the transgenic plant or transgenic plants produced
 from the transgenic plant seeds under conditions effective to enhance plant growth.

30 43. A method according to claim 42, wherein a transgenic plant is
 provided.

 44. A method according to claim 42, wherein a transgenic plant
 seed is provided.

 45. A method of insect control for plants comprising:

- 67 -

providing a transgenic plant or plant seed transformed with a DNA molecule which encodes a fragment of a hypersensitive response elicitor protein or polypeptide, which fragment does not elicit a hypersensitive response, and

5 growing the transgenic plant or transgenic plants produced
from the transgenic plant seeds under conditions effective to control insects.

46. A method according to claim 45, wherein a transgenic plant is provided.

10 47. A method according to claim 45, wherein a transgenic plant
seed is provided.

1/2

#1	HARPIN	
	<u>1</u>	
	403	
#3	C-TERMINAL FRAGMENTS	
#4	105	403
#5	169	403
#6	210	403
#7	267	403
	343	403
#8	N-TERMINAL FRAGMENTS	
#9	1	75
#10	1	104
#11	1	168
#12	1	266
	1	342
#13	INTERNAL FRAGMENTS	
#14	76	209
#15	76	168
#16	105	209
#17	105	169 209
	105	168
#18	SYNTHESIZED OLIGOPEPTIDES	
#19	99	209
#20	137	204
#21	137	180
#22	105	180
#23	150	209
	150	180

HARPIN FRAGMENTS DERIVED FROM HrpN OF ERWINIA AMYLOVORA

FIG. 1**SUBSTITUTE SHEET (RULE 26)**



2/2

N1; 5' -GGGAATTCATATGAGTCTGAATAACAGTGGG-3'
N76; 5' -GGGAATTCATATGGGCGGTGGCTAGGCGGT-3'
N99; 5' -GGCATATGTCGAACGCGCTGAACGATATG-3'
N105; 5' -GGGAATTCATATGTTAGGCGGTTCGCTGAAC-3'
N110; 5' -GGCATATGCTGAACACGCTGGGCTCGAAA-3'
N137; 5' -GGCATATGTCAACGTCCCACGACGAT-3'
N150; 5' -GGCATATGTCCACCTCAGACTCCAGCG-3'
N169; 5' -GGGAATTCATATGCAAAGCCTGTTGGTGATGGG-3'
N210; 5' -GGGAATTCATATGGGTAATGGTCTGAGCAAG-3'
N267; 5' -GGGAATTCATATGAAAGCGGGCATTCAAGGCG-3
N343; 5' -GGGAATTCATATGACACCAGCCAGTATGGAGCAG-3'
C75; 5' -GCAAGCTTAACAGCCCACCACCGCCCATCAT-3'
C104; 5' -GCAAGCTTAAATCGTTAGCGCGTTCAGCAG-3'
C168; 5' -GCAAGCTTAAATATCTCGCTGAACATCTTCAGCAG-3'
C180; 5' -GCAAGCTTAAAGGTGCCATCTGCCATCAC-3'
C204; 5' -GCAAGCTTAAATCAGTGACTCCTTTTATAGGC-3
C209; 5' -GCAAGCTTAAACAGGCCGACAGCGCATCAGT-3'
C266; 5' -GCAAGCTTAAACCGATACCGGTACCCACGGC-3'
C342; 5' -GCAAGCTTAAATCCGTCGTATCTGGCTTGCTCAG-3'
C403; 5' -GCAAGCTTAAAGCCCGCCAGCTTG-3'

OLIGONUCLEOTIDE PRIMERS USED FOR THE CONSTRUCTION
OF THE SUBCLONES OF *ERWINIA AMYLOVORA* HrpN

FIG. 2



SEQUENCE LISTING

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<151> 1998-10-05

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35

40

45

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50 55 60

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85 90 95

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290

295

300

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Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met
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Met Met Met Ser Met Met Gly Gly Gly Leu Met Gly Gly Gly Leu
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Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu
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Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr
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Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro
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Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln
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Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly
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Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu
 180 185 190



Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly
195 200 205

Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly
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225 230 235 240

Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln
245 250 255

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260 265 270

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290 295 300

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Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser
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<212> DNA

<213> Erwinia amylovora

<400> 25

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<210> 26

<211> 447

<212> PRT

<213> Erwinia amylovora

<400> 26

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Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala
 35 40 45

Gln Leu Leu Ala Glu Leu Leu Lys Ser Leu Leu Ser Pro Gln Ser Gly
 50 55 60

Asn Ala Ala Thr Gly Ala Gly Gly Asn Asp Gln Thr Thr Gly Val Gly
 65 70 75 80

Asn Ala Gly Gly Leu Asn Gly Arg Lys Gly Thr Ala Gly Thr Thr Pro
 85 90 95

Gln Ser Asp Ser Gln Asn Met Leu Ser Glu Met Gly Asn Asn Gly Leu
 100 105 110

Asp Gln Ala Ile Thr Pro Asp Gly Gln Gly Gly Gln Ile Gly Asp
 115 120 125

Asn Pro Leu Leu Lys Ala Met Leu Lys Leu Ile Ala Arg Met Met Asp
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Gly Gln Ser Asp Gln Phe Gly Gln Pro Gly Thr Gly Asn Asn Ser Ala
 145 150 155 160

Ser Ser Gly Thr Ser Ser Ser Gly Gly Ser Pro Phe Asn Asp Leu Ser
 165 170 175

Gly Gly Lys Ala Pro Ser Gly Asn Ser Pro Ser Gly Asn Tyr Ser Pro
 180 185 190



Val S r Thr Phe Ser Pro Pro Ser Thr Pr Thr Ser Pro Thr Ser Pr
 195 200 205

Leu Asp Phe Pro Ser Ser Pro Thr Lys Ala Ala Gly Gly Ser Thr Pro
 210 215 220

Val Thr Asp His Pro Asp Pro Val Gly Ser Ala Gly Ile Gly Ala Gly
 225 230 235 240

Asn Ser Val Ala Phe Thr Ser Ala Gly Ala Asn Gln Thr Val Leu His
 245 250 255

Asp Thr Ile Thr Val Lys Ala Gly Gln Val Phe Asp Gly Lys Gly Gln
 260 265 270

Thr Phe Thr Ala Gly Ser Glu Leu Gly Asp Gly Gly Gln Ser Glu Asn
 275 280 285

Gln Lys Pro Leu Phe Ile Leu Glu Asp Gly Ala Ser Leu Lys Asn Val
 290 295 300

Thr Met Gly Asp Asp Gly Ala Asp Gly Ile His Leu Tyr Gly Asp Ala
 305 310 315 320

Lys Ile Asp Asn Leu His Val Thr Asn Val Gly Glu Asp Ala Ile Thr
 325 330 335

Val Lys Pro Asn Ser Ala Gly Lys Ser His Val Glu Ile Thr Asn
 340 345 350

Ser Ser Phe Glu His Ala Ser Asp Lys Ile Leu Gln Leu Asn Ala Asp
 355 360 365

Thr Asn Leu Ser Val Asp Asn Val Lys Ala Lys Asp Phe Gly Thr Phe
 370 375 380

Val Arg Thr Asn Gly Gly Gln Gln Gly Asn Trp Asp Leu Asn Leu Ser
 385 390 395 400

His Ile Ser Ala Glu Asp Gly Lys Phe Ser Phe Val Lys Ser Asp Ser
 405 410 415

Glu Gly Leu Asn Val Asn Thr Ser Asp Ile Ser Leu Gly Asp Val Glu
 420 425 430

Asn His Tyr Lys Val Pro Met Ser Ala Asn Leu Lys Val Ala Glu
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<210> 27
<211> 5517
<212> DNA
<213> *Erwinia amylovora*

<400> 27

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<210> 28

<211> 1838

<212> PRT

<213> *Erwinia amylovora*

<400> 28

Met Glu Leu Lys Ser Leu Gly Thr Glu His Lys Ala Ala Val His Thr
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Ala Ala His Asn Pro Val Gly His Gly Val Ala Leu Gln Gln Gly Ser
 20 25 30

Ser Ser Ser Ser Pro Gln Asn Ala Ala Ala Ser Leu Ala Ala Glu Gly
 35 40 45

Lys Asn Arg Gly Lys Met Pro Arg Ile His Gln Pro Ser Thr Ala Ala
 50 55 60

Asp Gly Ile Ser Ala Ala His Gln Gln Lys Lys Ser Phe Ser Leu Arg
 65 70 75 80

Gly Cys Leu Gly Thr Lys Lys Phe Ser Arg Ser Ala Pro Gln Gly Gln
 85 90 95

Pro Gly Thr Thr His Ser Lys Gly Ala Thr Leu Arg Asp Leu Leu Ala
 100 105 110

Arg Asp Asp Gly Glu Thr Gln His Glu Ala Ala Ala Pro Asp Ala Ala
 115 120 125

Arg Leu Thr Arg Ser Gly Gly Val Lys Arg Arg Asn Met Asp Asp Met
 130 135 140

Ala Gly Arg Pro Met Val Lys Gly Gly Ser Gly Glu Asp Lys Val Pro
 145 150 155 160

Thr Gln Gln Lys Arg His Gln Leu Asn Asn Phe Gly Gln Met Arg Gln
 165 170 175

Thr Met Leu Ser Lys Met Ala His Pro Ala Ser Ala Asn Ala Gly Asp
 180 185 190

Arg Leu Gln His Ser Pro Pro His Ile Pro Gly Ser His His Glu Ile



195	200	205
Lys Glu Glu Pro Val Gly Ser Thr Ser Lys Ala Thr Thr Ala His Ala		
210	215	220
Asp Arg Val Glu Ile Ala Gln Glu Asp Asp Asp Ser Glu Phe Gln Gln		
225	230	235
240		
Leu His Gln Gln Arg Leu Ala Arg Glu Arg Glu Asn Pro Pro Gln Pro		
245	250	255
Pro Lys Leu Gly Val Ala Thr Pro Ile Ser Ala Arg Phe Gln Pro Lys		
260	265	270
Leu Thr Ala Val Ala Glu Ser Val Leu Glu Gly Thr Asp Thr Thr Gln		
275	280	285
Ser Pro Leu Lys Pro Gln Ser Met Leu Lys Gly Ser Gly Ala Gly Val		
290	295	300
Thr Pro Leu Ala Val Thr Leu Asp Lys Gly Lys Leu Gln Leu Ala Pro		
305	310	315
320		
Asp Asn Pro Pro Ala Leu Asn Thr Leu Leu Lys Gln Thr Leu Gly Lys		
325	330	335
Asp Thr Gln His Tyr Leu Ala His His Ala Ser Ser Asp Gly Ser Gln		
340	345	350
His Leu Leu Leu Asp Asn Lys Gly His Leu Phe Asp Ile Lys Ser Thr		
355	360	365
Ala Thr Ser Tyr Ser Val Leu His Asn Ser His Pro Gly Glu Ile Lys		
370	375	380
Gly Lys Leu Ala Gln Ala Gly Thr Gly Ser Val Ser Val Asp Gly Lys		
385	390	395
400		
Ser Gly Lys Ile Ser Leu Gly Ser Gly Thr Gln Ser His Asn Lys Thr		
405	410	415
Met Leu Ser Gln Pro Gly Glu Ala His Arg Ser Leu Leu Thr Gly Ile		
420	425	430
Trp Gln His Pro Ala Gly Ala Ala Arg Pro Gln Gly Glu Ser Ile Arg		
435	440	445
Leu His Asp Asp Lys Ile His Ile Leu His Pro Glu L u Gly Val Trp		

450 455 460
Gln Ser Ala Asp Lys Asp Thr His S r Gln Leu Ser Arg Gln Ala Asp
465 470 475 480
Gly Lys Leu Tyr Ala Leu Lys Asp Asn Arg Thr Leu Gln Asn Leu Ser
485 490 495
Asp Asn Lys Ser Ser Glu Lys Leu Val Asp Lys Ile Lys Ser Tyr Ser
500 505 510
Val Asp Gln Arg Gly Gln Val Ala Ile Leu Thr Asp Thr Pro Gly Arg
515 520 525
His Lys Met Ser Ile Met Pro Ser Leu Asp Ala Ser Pro Glu Ser His
530 535 540
Ile Ser Leu Ser Leu His Phe Ala Asp Ala His Gln Gly Leu Leu His
545 550 555 560
Gly Lys Ser Glu Leu Glu Ala Gln Ser Val Ala Ile Ser His Gly Arg
565 570 575
Leu Val Val Ala Asp Ser Glu Gly Lys Leu Phe Ser Ala Ala Ile Pro
580 585 590
Lys Gln Gly Asp Gly Asn Glu Leu Lys Met Lys Ala Met Pro Gln His
595 600 605
Ala Leu Asp Glu His Phe Gly His Asp His Gln Ile Ser Gly Phe Phe
610 615 620
His Asp Asp His Gly Gln Leu Asn Ala Leu Val Lys Asn Asn Phe Arg
625 630 635 640
Gln Gln His Ala Cys Pro Leu Gly Asn Asp His Gln Phe His Pro Gly
645 650 655
Trp Asn Leu Thr Asp Ala Leu Val Ile Asp Asn Gln Leu Gly Leu His
660 665 670
His Thr Asn Pro Glu Pro His Glu Ile Leu Asp Met Gly His Leu Gly
675 680 685
Ser Leu Ala Leu Gln Glu Gly Lys Leu His Tyr Phe Asp Gln Leu Thr
690 695 700
Lys Gly Trp Thr Gly Ala Glu Ser Asp Cys Lys Gln Leu Lys Lys Gly

705	710	715	720
Leu Asp Gly Ala Ala Tyr Leu Leu Lys Asp Gly Glu Val Lys Arg Leu			
725	730	735	
Asn Ile Asn Gln Ser Thr Ser Ser Ile Lys His Gly Thr Glu Asn Val			
740	745	750	
Phe Ser Leu Pro His Val Arg Asn Lys Pro Glu Pro Gly Asp Ala Leu			
755	760	765	
Gln Gly Leu Asn Lys Asp Asp Lys Ala Gln Ala Met Ala Val Ile Gly			
770	775	780	
Val Asn Lys Tyr Leu Ala Leu Thr Glu Lys Gly Asp Ile Arg Ser Phe			
785	790	795	800
Gln Ile Lys Pro Gly Thr Gln Gln Leu Glu Arg Pro Ala Gln Thr Leu			
805	810	815	
Ser Arg Glu Gly Ile Ser Gly Glu Leu Lys Asp Ile His Val Asp His			
820	825	830	
Lys Gln Asn Leu Tyr Ala Leu Thr His Glu Gly Glu Val Phe His Gln			
835	840	845	
Pro Arg Glu Ala Trp Gln Asn Gly Ala Glu Ser Ser Trp His Lys			
850	855	860	
Leu Ala Leu Pro Gln Ser Glu Ser Lys Leu Lys Ser Leu Asp Met Ser			
865	870	875	880
His Glu His Lys Pro Ile Ala Thr Phe Glu Asp Gly Ser Gln His Gln			
885	890	895	
Leu Lys Ala Gly Gly Trp His Ala Tyr Ala Ala Pro Glu Arg Gly Pro			
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Leu Ala Val Gly Thr Ser Gly Ser Gln Thr Val Phe Asn Arg Leu Met			
915	920	925	
Gln Gly Val Lys Gly Lys Val Ile Pro Gly Ser Gly Leu Thr Val Lys			
930	935	940	
Leu Ser Ala Gln Thr Gly Gly Met Thr Gly Ala Glu Gly Arg Lys Val			
945	950	955	960
Ser Ser Lys Phe Ser Glu Arg Ile Arg Ala Tyr Ala Phe Asn Pro Thr			

965

970

975

Met Ser Thr Pro Arg Pr Ile Lys Asn Ala Ala Tyr Ala Thr Gln His
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Gly Trp Gln Gly Arg Glu Gly Leu Lys Pro Leu Tyr Glu Met Gln Gly
 995 1000 1005

Ala Leu Ile Lys Gln Leu Asp Ala His Asn Val Arg His Asn Ala Pro
 1010 1015 1020

Gln Pro Asp Leu Gln Ser Lys Leu Glu Thr Leu Asp Leu Gly Glu His
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Gly Ala Glu Leu Leu Asn Asp Met Lys Arg Phe Arg Asp Glu Leu Glu
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Gln Ser Ala Thr Arg Ser Val Thr Val Leu Gly Gln His Gln Gly Val
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Leu Lys Ser Asn Gly Glu Ile Asn Ser Glu Phe Lys Pro Ser Pro Gly
 1075 1080 1085

Lys Ala Leu Val Gln Ser Phe Asn Val Asn Arg Ser Gly Gln Asp Leu
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Ser Lys Ser Leu Gln Gln Ala Val His Ala Thr Pro Pro Ser Ala Glu
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Ser Lys Leu Gln Ser Met Leu Gly His Phe Val Ser Ala Gly Val Asp
 1125 1130 1135

Met Ser His Gln Lys Gly Glu Ile Pro Leu Gly Arg Gln Arg Asp Pro
 1140 1145 1150

Asn Asp Lys Thr Ala Leu Thr Lys Ser Arg Leu Ile Leu Asp Thr Val
 1155 1160 1165

Thr Ile Gly Glu Leu His Glu Leu Ala Asp Lys Ala Lys Leu Val Ser
 1170 1175 1180

Asp His Lys Pro Asp Ala Asp Gln Ile Lys Gln Leu Arg Gln Gln Phe
 1185 1190 1195 1200

Asp Thr Leu Arg Glu Lys Arg Tyr Glu Ser Asn Pro Val Lys His Tyr
 1205 1210 1215

Thr Asp Met Gly Phe Thr His Asn Lys Ala L u Glu Ala Asn Tyr Asp



1220	1225	1230
Ala Val Lys Ala Phe Ile Asn Ala Phe Lys Lys Glu His His His Gly Val		
1235	1240	1245
Asn Leu Thr Thr Arg Thr Val Leu Glu Ser Gln Gly Ser Ala Glu Leu		
1250	1255	1260
Ala Lys Lys Leu Lys Asn Thr Leu Leu Ser Leu Asp Ser Gly Glu Ser		
1265	1270	1275
Met Ser Phe Ser Arg Ser Tyr Gly Gly Val Ser Thr Val Phe Val		
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Pro Thr Leu Ser Lys Lys Val Pro Val Pro Val Ile Pro Gly Ala Gly		
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Ile Thr Leu Asp Arg Ala Tyr Asn Leu Ser Phe Ser Arg Thr Ser Gly		
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Gly Leu Asn Val Ser Phe Gly Arg Asp Gly Gly Val Ser Gly Asn Ile		
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Met Val Ala Thr Gly His Asp Val Met Pro Tyr Met Thr Gly Lys Lys		
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Thr Ser Ala Gly Asn Ala Ser Asp Trp Leu Ser Ala Lys His Lys Ile		
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Ser Pro Asp Leu Arg Ile Gly Ala Ala Val Ser Gly Thr Leu Gln Gly		
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Thr Leu Gln Asn Ser Leu Lys Phe Lys Leu Thr Glu Asp Glu Leu Pro		
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Gly Phe Ile His Gly Leu Thr His Gly Thr Leu Thr Pro Ala Glu Leu		
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1440		
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Leu Asn Glu Asp Gly Ser Lys Pro Asn Gly Val Thr Ala Arg Val Ser		
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Ala Gly Leu Ser Ala Ser Ala Asn Leu Ala Ala Gly Ser Arg Glu Arg		



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Pro Val Thr Ser Asn Asp Ile Ser Glu Leu Thr Ser Thr Leu Gly Lys		
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His Phe Lys Asp Ser Ala Thr Thr Lys Met Leu Ala Ala Leu Lys Glu		
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1715	1720	1725
Gln Thr Glu Lys Ala Ile Leu Asp Gly Lys Val Gly Arg Glu Glu Val		



1730

1735

1740

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 1745 1750 1755 1760

Val Ser Gln Ser Val Ser Lys Ser Glu Gly Phe Asn Thr Pro Ala Leu
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Gly Thr Ile Asn Phe Lys Tyr Gly Gln Asp Gln Asn Thr Pro Arg Arg
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<212> DNA

<213> Erwinia amylovora

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 cagcagtcgc tggagcatct ggatgaagca agtttttagcg atatcgtag cggcttcatc 360
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<213> Erwinia amylovora

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35

40

45

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Thr Ser Ile Thr Leu Tyr Ser Met Leu Leu Gln Leu Asn Phe Glu Met
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Arg Leu Cys Phe Gln Gln Ser Leu Glu His Leu Asp Glu Ala Ser Phe
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<210> 31

<211> 341

<212> PRT

<213> Pseudomonas syringae

<400> 31

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Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala
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Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val
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Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
 115 120 125

Thr Lys Gln Asp Gly Gly Thr Ser Ph Ser Glu Asp Asp Met Pro Met
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Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe
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 195 200 205

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Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Leu Gly Thr Pro Val
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Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln
 275 280 285

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 290 295 300

Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala
 305 310 315 320

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<210> 32
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<212> DNA

<213> *Pseudomonas syringae*

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<210> 33

<211> 1729

<212> DNA

<213> *Pseudomonas syringae*

<400> 33

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<210> 34

<211> 424

<212> PRT

<213> *Pseudomonas syringae*

<400> 34

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Glu	Gln	Asn	Thr	Gln	Gln	Ala	Ile	Asp	Pro	Ser	Ala	Leu	Leu	Phe	Gly
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Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln		
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Lys Phe Ala Leu Val Lys Ser Asp Ser Asp Asp Leu Lys Leu Ala Thr		
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Ala Ser Thr Gln His Thr Glu Leu		



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<210> 35

<211> 344

<212> PRT

<213> *Pseudomonas solanacearum*

<220>

<223> Description of Unknown Organism: *Pseudomonas solanacearum*

<400> 35

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35 40 45Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly
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85 90 95Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met
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115 120 125Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val
130 135 140Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala
145 150 155 160Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly
165 170 175Gly Ala Gly Ala Gly Gly Ala Gly Gly Val Gly Gly Ala Gly Gly
180 185 190



Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Asn Gly Ala
 195 200 205

Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn
 210 215 220

Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp
 225 230 235 240

Gln Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn
 245 250 255

Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Asn Gln
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Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly
 275 280 285

Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser
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<210> 36

<211> 1035

<212> DNA

<213> *Pseudomonas solanacearum*

<400> 36

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<211> 26

<212> PRT

<213> *Xanthomonas campestris* pv. *glycines*

<400> 37

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<210> 38

<211> 20

<212> PRT

<213> *Xanthomonas campestris* pv. *pelargonii*

<400> 38

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<210> 39

<211> 13

<212> PRT

<213> *Phytophthora megasperma*

<400> 39

Val	Trp	Asn	Gln	Pro	Val	Arg	Gly	Phe	Lys	Val	Tyr	Glu			
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INTERNATIONAL SEARCH REPORT

Int'l Application No
PCT/US 99/23181

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/195 C12N15/31
A01H5/10 C12N15/82

C12N1/21

C12N5/10

A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NÜRNBERGER T, ET AL. : "High Affinity Binding of a Fungal Oligopeptide Elicitor to arsley Plasma Membranes Triggers Multiple Defense Responses" CELL, vol. 78, no. 3, 12 August 1994 (1994-08-12), pages 449-460, XP000882736 Cambridge, Mass. cited in the application the whole document	1, 2, 10, 11, 19-23, 30-32, 36-38
A	WO 98 32844 A (CORNELL RES FOUNDATION INC) 30 July 1998 (1998-07-30) the whole document	—/—

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the International filing date
- "L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the International filing date but later than the priority date claimed

"T" later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

6 March 2000

Date of mailing of the international search report

03/04/2000

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Bilang, J

INTERNATIONAL SEARCH REPORT

Int'l. Application No
PCT/US 99/23181

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 24297 A (CORNELL RES FOUNDATION INC) 11 June 1998 (1998-06-11) the whole document	
A	WEI Z-M, ET AL.: "Harpin, an HR elicitor, activates both defense and growth systems in many commercially important crops" PHYTOPATHOLOGY, vol. 88, September 1998 (1998-09), page S96 XP000882741 abstract	
A	NIGGEMEYER J, ET AL.: "Characterization of the functional domains of harpin" PHYTOPATHOLOGY, vol. 88, September 1998 (1998-09), page S67 XP000882740 abstract	

INTERNATIONAL SEARCH REPORT
Information on patent family membersIndex Application No
PCT/US 99/23181

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9832844	A 30-07-1998	AU	6043198 A	18-08-1998
WO 9824297	A 11-06-1998	AU EP	5693598 A 0957672 A	29-06-1998 24-11-1999

